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The Yale Journal of Biology & Medicine

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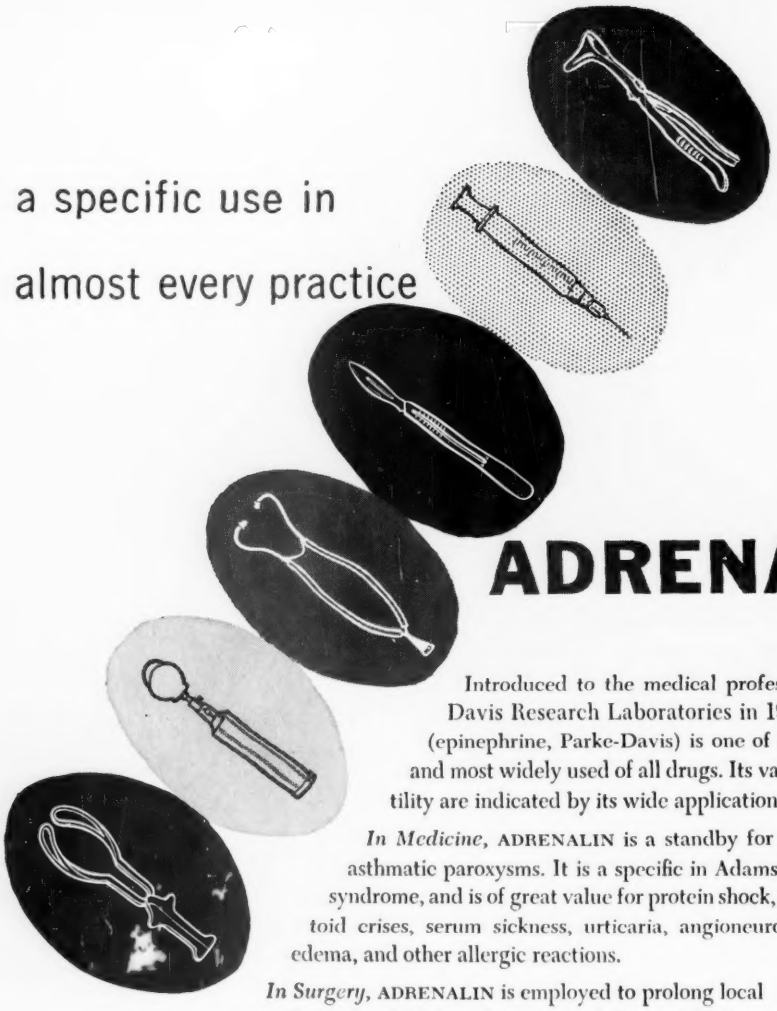

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THE YALE JOURNAL
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ERRATUM: The Editors regret that two errors appeared in the April 1953 issue of the Journal in the paper by Hale and Stoner on "The effect of cobalt-60 gamma radiation on passive immunity." In the sentence at the bottom of page 326, "LD_{50} for mice twenty-eight days of age was 750 Rep per hour," the words "per hour" should be deleted. In the third line from the bottom of page 331 "these antibodies" should read "these antibiotics."

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SAMUEL CLARK HARVEY

From a portrait by Deane Keller presented to Yale University in 1951 by the surgeons who had served as residents from 1922 until 1947, the tenure of Dr. Harvey's chairmanship of the Department of Surgery.

MAX TAFFEL

Department of Surgery, Yale University School of Medicine

SAMUEL CLARK HARVEY, 1886-1953

On Saturday morning, 22 August 1953, Dr. Harvey arrived at his usual hour in his study on the balcony of the Historical Library. He had come to work on a paper on wound healing that he was preparing for presentation at the coming meeting of the International Society of Surgery at Lisbon. It was a radiant summer's day and he was alone not only in the sanctum of his study but in all the Library, which is closed on Saturdays during the summer holidays. There, in the midst of the books he loved and treasured, Dr. Harvey died. The end apparently came with lightning swiftness.

He died in the manner that he would have chosen for himself—quietly and unobtrusively, while in full possession of his physical and mental faculties, in the silent company of kindred masters and sages of many centuries, and, above all, while actively engaged in productive scientific effort.

Thus, in one shattering moment, there came to an end a distinguished career and a singularly rich and noble life that had been steeped in the finest traditions of scholarship and that had been dedicated to the alleviation of pain and suffering, which are the common heritage of all mankind. His passing is too recent and the heart is too full to write about him without emotion. He, however, would not have wanted, even after death, any indulgence in emotion on his behalf or any public bestowal of garlands of praise, no matter how richly they had been deserved or how sincerely and understandingly they had been proffered. Perhaps he would not mind if, for historical record, the annals of his life were set down in simple and unadorned language and if they were permitted to speak for themselves and to show the indelible impress that he made in so many varied fields of human endeavor and upon all who had the good fortune to know him and to come within the sphere of his influence.

Born on 12 February 1886, on a small farm in Washington, Connecticut, Samuel Harvey spent much of his boyhood helping his father eke out a precarious sustenance from Connecticut's rocky earth. He never lost his love for farm life and in later years often expressed the hope that he might some day be able to go back to the place of his birth and till the soil as his ancestors had done before him. His formal education began in a one-room country school in Woodbury, and his teacher, who is still alive, relates that his keen intellect and inquisitive mind were evident even in those early

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years. He attended the Woodbury High School and the Mount Hermon School, and then entered Yale College, graduating with the class of 1907. His first interests lay in the field of engineering, but in the course of his college career they shifted to biology and then to medicine.

Of the fifty-three students who matriculated in the Yale Medical School in 1907, Samuel Harvey was one of only four with a baccalaureate degree. He made an outstanding record, receiving honorable mention for the anatomical prize at the end of his second year, sharing with a classmate the Keese prize for the most meritorious thesis in his senior year, and receiving his M.D. degree *cum laude*. Despite the taxing schedule in medical school, he found time to be secretary and president of his class, to edit the class yearbook, and to serve as student editor of the *Yale Medical Journal*. His talent for original investigation also came to light at that time, and, when he was graduated in 1911, he had already published five significant scientific papers. In the first, published in his twenty-first year, he reported the isolation of an organism from chicks which had died on his father's farm, and confirmed Rettger's discovery that the *Salmonella pullorum* was the causative agent of an epidemic and fatal septicemia in young chickens. Three papers dealt with the still unsolved problem of "shock," and in the fifth he described an original quantitative method, widely employed to this day, for determining chlorides in the urine.

Following graduation from medical school he went to New York City and spent two years with Professor William G. MacCallum, in the Department of Pathology at the College of Physicians and Surgeons. In common with many pathologists of those days, he contracted an open pulmonary apical lesion and was compelled to retire to the Loomis Sanatorium where he spent one year "curing" his own disease and serving on the staff as assistant resident physician. In 1914 he was awarded a coveted appointment on Dr. Harvey Cushing's surgical service at the Peter Bent Brigham Hospital in Boston. Here he received his first indoctrination in the art and science of surgery and spent three crowded and stimulating years as a house officer (1914-1915), Arthur Tracy Cabot Fellow (1915-1916), and assistant resident surgeon (1916-1917).

Throughout these years of postgraduate training, when his time was almost completely consumed by the traditionally pressing duties of surgical intern and assistant resident, his investigative interests did not lie fallow. Among other studies, he continued his researches on "shock"; he published an anatomical study on the Malpighian bodies in nephritis; he accurately described the disposition of congenital bands in the region of the vermiform

appendix, dispelling conclusively and with finality a prevalent misconception that they were inflammatory in origin; and, as far back as 1916, he experimented with absorbable fibrin paper as a hemostatic agent, a precursor of the recently developed oxidized gauze, fibrin foam, and gelatin foam, without which modern surgeons would be severely handicapped.

In 1917, at the height of World War I, he interrupted his formal surgical training to join the newly organized U.S. Army Base Hospital No. 5 under the direction of Dr. Cushing. He went overseas with this unit in May 1917 and served in France for almost two years, first with the British and later with the American Expeditionary Forces. In recognition of his superior qualities of leadership and of his skill as a neurosurgeon, Dr. Cushing appointed him chief of surgery in Mobile Hospital No. 6, where he was responsible for the care of large numbers of neurosurgical casualties, often operating without respite for more than forty-eight hours at a time under difficult and improvised field conditions. At the termination of the war, the enlisted men of his organization, then uninhibited by military discipline, wrote their own history of Base Hospital No. 5 and, with the notorious perspicacity and often ruthless yet sympathetic candor of enlisted men, described the virtues and foibles of their officers. It may not be irreverent to quote here from their "citation" for Dr. Harvey, for in a few simple and pithy sentences they seemed to have caught the flavor of the man. "There are five things that Sam is never without: a cigar or a pipe, a book, a desire to stay longer in bed, wonderful good humor, and clear straight thinking. This officer has settled more problems by quiet reflection than by the experimental method of trying them out, and there is much to say in favor of this method as an energy-saving way. [His] greatest success in the affairs of the unit was his appearance on the stage, where his natural talent as a comedian won him instant vociferous acclamation. He has many other claims to which we cannot do justice, but besides being a most all-round surgeon, he is a walking encyclopedia of all sorts."

Once again a civilian, he returned in 1919 to become resident surgeon at the New Haven Hospital for one year. He was appointed Assistant Professor of Surgery in the Yale Medical School in 1920 and promoted to Associate Professor and Acting Chairman of the Surgical Department in 1921. In 1924 his alma mater conferred upon him one of her highest awards and made him a full professor and chairman of the department, a post he held with one brief interruption until 1947.

It was during the years of his professorship that he rose to his full stature. Following Halsted's precepts, he quickly developed a residency-training program, which from the very beginning was acknowledged to be

of the first rank and which attracted graduates of high caliber from medical schools throughout the country. Ready at all times to place his knowledge at their disposal and to advise them out of his learning and experience, he expected from his house officers and residents the highest standards of performance. In his gentle manner he exerted restraint where necessary, but he was never failing in understanding and encouragement.

He approached the study of disease in the true spirit of scientific inquiry, insisting upon accurate observations and logical and warranted conclusions. Intimately familiar with all branches of surgery, he exemplified in its broadest sense the type of "general surgeon" which was rare enough even in his day, but which in this age of ultra-specialization has all but vanished from the surgical scene. In addition to a remarkable versatility in abdominal and peripheral surgery, he was one of the pioneers in this country in neurosurgery, as well as thoracic and vascular surgery, operating with equal facility upon the central nervous system, the lungs, the mediastinum, and the heart and its great vessels. He operated quietly, steadily, and deftly, without haste but also without wasted motions. His operations were not merely demonstrations of superior craftsmanship and manual dexterity; they were more in the nature of physiological experiments, thoughtfully conceived, carefully planned, and solidly founded upon a wide knowledge of the basic sciences.

In the operating room, as indeed everywhere else, he exhibited an imperturbability that was envied and zealously emulated, though not always with success, by his colleagues and house staff. He never raised his voice and rarely lost his temper. When the problem at hand was particularly trying or when he had reached the limit of his patience with his assistants or his nurse, he would turn away from the operating table, splash his hands vigorously for a few moments in the sterile wash basin behind him, and then return to the table. This was the only indulgence he permitted himself. He felt a deep and sympathetic concern for his patients and was ever conscious of the enormous trust which, with implicit and unquestioning faith, they had placed in his hands. But, even during critical phases of their illness, he rarely permitted his anxiety to reach the visible surface, and only those who knew him well could detect in his manner a faint betrayal of worry and apprehension.

In his philosophy of pedagogy he was far ahead of his time and was to a large extent responsible for the introduction of the unique method of teaching that is colloquially referred to as the "Yale system." He regarded the medical student as a mature adult who entered medical school with a sincere desire to learn, and he placed the responsibility for his education

squarely upon the student himself rather than upon the faculty. A staunch and outspoken antagonist of the didactic lecture and quiz method of teaching, he believed that knowledge cannot be passively transferred or given away, but can be acquired only by an active, painstaking, and continuing process of discriminating study and reflection. He considered it the function of the teacher to arouse enthusiasm and stimulate interest in the students, to provide them with opportunities for pursuing these interests, to guide them in the use of the "experimental method," to help them develop habits of effective and sustained study—but above all to spark their curiosity and to develop within them a rational spirit of inquiry that would be a stimulus for learning and study long after they had left the protective aegis of school and teachers.

In research, Dr. Harvey's mind was constantly directed to a wide variety of timely and important subjects, not only in nearly every branch of surgery, but in many facets of medical history, education, and socio-economics. His most outstanding experiments, now considered classic, were on the subject of wound healing. He devoted many years to exploring every phase of this phenomenon, searching not just for significant correlative factors, but mining more deeply into the fundamental mechanisms that determined, in such a constant and orderly manner, the reproduction, growth, and differentiation of the fibroblast, which is the basic cell in the healing wound. From his experimental studies, in the clinic but more particularly in the laboratory, have come many solid and original contributions to knowledge. His philosophical and historical essays, written in an easy and homely style, have a quality of timelessness about them, and reveal the breadth of his erudition, the clarity of his logic, and the soundness of his thinking.

The many years of intensive study, teaching, experimental investigation, and heavy clinical and operating duties did not isolate him from other responsibilities. His steadying influence was continuously felt in many important policy-making committees in the Medical School. A member of the founder's group, he served for seven years on the American Board of Surgery, introducing his concepts of post-doctorate education in surgery and traveling to all parts of the country to examine candidates for certification. In further recognition of his interest in the training of young surgeons, he was elected to the board of governors of the American College of Surgeons in 1936. He was an editor of some of the more illustrious surgical journals. He belonged to numberless medical and scientific societies, and was honored by election to the presidency of several of the oldest and most renowned of them. He maintained a lively and active interest in the affairs of the Connecticut State Medical Society and served as its president for

one year. Generally regarded as a sage, his counsel and advice were constantly sought on all manner of things. No matter how complex the problem or in what channel of learning or human activity it lay, he had a remarkable faculty for culling out the pertinent facts, getting at once to the heart of the situation, and introducing light and encouragement where only darkness and often despair had existed before.

In 1936, a flare-up of his pulmonary lesion seriously threatened to end his active academic career. Once again he went to a sanatorium, but after one year he felt well enough to return to his post and resume his duties, working at first for only a few hours in the day, but quickly adding to them until he was back at his old pace.

During World War II, when nearly all the members of his department were away in the armed forces, he was heavily burdened with routine duties and with the added teaching responsibilities that came with the increase in the size of the classes and with the year-round schedule of the accelerated program. He strove valiantly to keep his department together and to maintain the same high standards in the face of an ever-dwindling faculty and house staff. In addition to all this, he served on the surgical committee of the National Research Council, spending many precious hours at meetings and in travel to and from Washington, D. C.

He relinquished the chairmanship of the Surgical Department in 1947, became Professor of Surgery (Oncology) and began to devote himself exclusively to the study of malignant neoplastic disease. In 1949, he was stricken for the third time by a serious illness and, consistent with a common tradition among surgeons, was operated upon for the same malady in which he himself was then intensely interested and to the understanding of which he had made many signal contributions. He made a quick and full recovery, but he decided to taper off his heavy clinical schedule, and, in 1950, retired also from his active professorship in oncology.

His retirement did not, however, signify any slackening of pace. He shifted his base from the Department of Surgery to the Historical Library of the Medical School and, never losing his buoyancy, pursued his scholarly and other activities until the last moment. He took on the thankless task of medical procurement officer in Connecticut for the Armed Forces. He devoted many hours of faithful service to the Gaylord Farm Sanatorium, where he had been a member of the governing board for many years. He accepted the editorship of *The Yale Journal of Biology and Medicine* and applied himself with energy and fervor, taking especial interest in the student editors, and characteristically being more daring and progressive in his ideas than his younger colleagues on the Board. He continued his

research on burns, wrote two sections on surgery for the *Encyclopedia Britannica,* gathered source material for a history of surgery, and was in the process of preparing a philosophical treatise on the fundamental aspects of wound healing when his end came.

No bare recital of a curriculum vitae can adequately reveal his heart and mind. He was a man of uncommon culture and refinement. He plowed deeply into many diverse fields of learning and went through life constantly studying and observing. He read widely and rapidly and with discernment, cataloguing, without seeming effort, everything he read in a phenomenally retentive memory. His labyrinthine mind was rich in stores of knowledge and wisdom, held in a modest and almost shy reserve. He was uncannily shrewd in his observations concerning people, and he seemed to have the prescience of a prophet in his speculations on events to come. All things interested him. He warmed his hands at many fires of life and he always tossed in a few coals from his own wisdom to make the flames grow brighter. Scholar, teacher, scientist, philosopher, wit, historian, economist, counsellor, healer of the sick, delightful companion, devoted and generous friend, he was a man of truly Leonardian stature. Being mortal, he was not spared his full portion of sorrow and sadness, and these he bore with quiet dignity and humility.

Time alone determines the ultimate measure of a man and appoints his proper place in the long pilgrimage of those who, throughout the ages, have dedicated their lives to the pursuit of truth and to the service of mankind. Dr. Harvey's genius and greatness are acknowledged by all his contemporaries. The annals of his life make it abundantly clear that posterity will confirm this judgment.

SAMUEL C. HARVEY

THE OBJECTIVES OF MEDICAL EDUCATION,
An Introduction to the Consideration of a Curriculum*

If one considers what the majority of the graduates of our medical schools do in their professional lives, the answer is, of course, that which is termed "general practice." Therefore, the objective of a medical education, it is customarily assumed, is that which will develop the best general practitioner. Of course, the qualities required of him are well recognized, but their relative importance may be a matter for argument; certainly the effectiveness of education upon certain of these is, to a considerable degree, still a matter of opinion.

The quality which every one would place first is that of integrity, both in the usual sense of honesty and also in the broader implications of its Latin derivation, of "wholeness and uprightness." It would also be agreed that this is a peculiarly desirable objective, whatever activity the physician might undertake in subsequent life. That which medical education can do about this on the positive side is, however, all too little. The student in the school of medicine is an adult, contrary to common opinion, and particularly so in respect to the underlying qualities of behavior and conduct. He has already been heavily conditioned by his environment in family, school, and college during those plastic and formative years when these qualities are most readily molded. In a negative sense and indirectly the school can do something by culling out at the start those who are obviously dishonest and by restricting admissions to those men whose *primary* interest in medicine is an intellectual one rather than one of advancement in a social or financial way. Once in, such changes as may occur for the better or for the worse will result from the example set by members of the medical profession within and without the faculty. The school can, at least, see to it that its faculty contains no member whose integrity in the broadest sense is questionable.

A second objective desired in the ideal physician is that of intelligence, or, better, a proper intellectual ability. Again, every one would agree that

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there must be in a student a basic capacity for learning, but many schools set this very low or indulge in the vain hope that by proper teaching this can be increased to a reasonable level. The latter is not true, in theory or in practice, though the learning habits of work may serve to bring a proper intelligence into action. This occasional occurrence leads to the false hope that many of those giving past evidence of low intelligence accomplishment may be regenerated in the medical school. It is time that schools of medicine stop admitting these, even if this does reduce the size of the student body and the resultant income from tuition.

A third quality, just referred to, is that of capacity for work, and by this is meant, for the most part, not physical but intellectual labor. In this there are two components, one a faculty for sustained work, the other, of no less importance, a faculty for initiating work. These may, for the sake of brevity, be called work endurance and work initiative, respectively. The lack of these in the physician leads to a static attitude where only the acute pressure of circumstances may occasionally force him out of a routine approach, learned in school as a finished thing. Probably the lack of initiative and the absence of a sufficiently demanding intellectual curiosity is responsible for more poor practice of medicine than any other one thing. The ideal physician will be dynamic, continually learning throughout his professional life by virtue of a capacity for work initiative and work endurance.

In relation to this the teaching methods of the school of medicine may and do accomplish much. It is here that the traditional theory of discipline enters in—so strongly that in university circles it is customary to refer to fields of study as disciplines." Behind this lies the concept that a timed, enforced performance will bring a student into habits of behavior that will persist throughout life and from this has arisen the educational method of enforced attendance at exercises, the controlled systematic coverage of material, the frequent checking by oral and written examinations, and the evaluation, in mathematical terms carried to absurd degrees, of the work done. The student puts his tuition in the slot, jumps in the hopper, and comes out the spout of the machine, a link of sausage even down to the cellophane wrapping of an appropriate degree. That the method is effective cannot be seriously questioned but that the effect is beneficial in respect to the continuing quality of work which the ideal physician should do is another thing, for work so done by command and rote, and so checked, destroys initiative and makes by these associations intellectual work disagreeable. The student becomes dependent upon a continuing obligatory supervision which will be conpicuously absent in his future professional life.

The only situation where such a form of discipline is carried out to a greater degree than in our present educational system is in the armed forces where a primary objective is unquestioning obedience to an always present command. Under these circumstances this is desirable, but in the absence of authoritative leadership such disciplined forces are bewildered and ineffective. So undesirable is it in other respects that the English language has been enriched by the word "soldiering," a behavior that is by no means unknown in disciplinary education.

Another quality that is highly desirable in the physician is that of "common sense" or, to descend further into the vernacular, "horse sense." Based on superficial empiricism or obtained *ex cathedra* from the teacher, this is frequently misleading and dangerous. On the other hand, when derived from a properly proportioned integration of one's knowledge and experience, it is of the greatest importance. Without "surgical judgment" the surgeon is likely to be a dangerous man, and is the more dangerous the greater his facility in technique, and while not so immediately apparent, this is equally true in the other divisions of medicine. That this quality can be taught by disciplinary methods is highly questionable, for its development depends upon a balanced integration of past experience and present situations, with a view to the future; a process which is learned for the most part by experience and example. Judgment is closely allied to that other abstract quality, integrity. With respect to both of these the student frequently is irretrievably conditioned by his family and scholastic environment before reaching the medical school. The best that the members of the faculty can do is to set an example in their own behavior while giving the student the opportunity to exercise his own judgment in situations involving responsibility so that he can learn by experience. Without responsibility involving an intrinsic reward or penalty for good or bad judgment, the exercise is largely meaningless.

Lastly, a quality which is basic in the properly educated physician is that of a faculty for ascertaining the truth (perhaps more strongly expressed in the German "die Wahrheit") or as close an approximation to it as is possible. Knowledge which does not correspond with reality is peculiarly dangerous in the field of medicine and by experience we know that even at its best it is of a changing quality. The ideal physician is not one who has a content of knowledge of a certain vintage, but one who has that of the present moment critically evaluated. Moreover, he is faced constantly, whether in the laboratory or the clinic, by problems soluble only by the "scientific method." Science defined as knowledge and art in the use of it are alike, based soundly only upon the scientific method which alone is constant while

that with which it deals is changing. It matters not whether the problem is confronted in the foundation sciences or in clinical medicine, whether in the laboratory or in the clinic, whether it be with the experimental animal or with the patient, the approach, although the nomenclature may be different, is the same. In the laboratory one accumulates related facts within a field of interest, and by the logical process of induction arrives at hypotheses which one tests by experience or experimentation. From this a further hypothesis may be constructed, and this process may go on indefinitely, in each phase arriving at a closer approximation to the truth. This method was first developed in clinical medicine, but with a terminology of its own. Here, the assembling of facts is the obtaining of subjective and objective data about the patient and from this is derived by induction a tentative diagnosis. From this one concludes by deduction that if this be correct, certain other facts must obtain, which are checked by further observation, or by experimentation, one form of which is treatment. The *art* of the clinician, as well as that of the investigator in the laboratory, is skill in the application of the scientific method; otherwise it is but a vain and deceiving quality. The scientific method is then a basic, one might well say, *the* basic motif underlying all learning in the medical school and about this its methodology of teaching should be oriented. To this, if achieved, all else will be added, at least in so far as teaching will do so.

It must be obvious that the scientific method cannot be properly taught by the lecture, or from the book. It is not a static thing such as is knowledge at a given moment, but a dynamic or functional process which is best learned by direct experience. The province of the faculty, clinical or otherwise, is to set an example by actively engaging themselves in the solution of problems by this method and in giving the student ample opportunity to do so himself, for with repeated use of any method comes skill.

It seems apparent, then, that of these qualities of integrity, intelligence, capacity for work, judgment, and skill in the use of the scientific method, none can be best taught by the traditional didactic and obligatory methods. They must all be learned by the student from example and by personal experience gained in the laboratory and the clinic, rather than in the lecture hall and the recitation room. Bring him into working contact with a senior person of integrity, of high intelligence, of great capacity for initiating and sustaining work, of sound judgment, and one constantly employing the scientific method in the solution of problems, and a sound approach will have been made toward the objectives which the school of medicine should have in view.

Many objections will be raised to this thesis, some based upon traditional and fixed concepts, some upon the supposed necessity for teaching masses of students of mediocre quality, and some upon misunderstanding. The last should be corrected in so far as one can foresee the points at issue.

It is notable in the discussion so far that the content of knowledge necessary for the medical student has not been referred to; and this has been intentional, for with a proper educational approach knowledge will be obtained not only during the school experience but as a continuing process thereafter. On the other hand, knowledge gained perforce from the teacher and under the system referred to as the disciplinary type of education has a fixed and dated content, and is of an authoritative and dogmatic nature which inhibits further development on the part of the student. One trained with the primary objective of a content of knowledge imparted from above, under rigid supervision and systematized instruction during a period of four years, is likely to have acquired a revulsion against the learning of knowledge. Particularly is this true of the average man, and it is a credit to our more intellectual students that so many have succeeded in circumventing this deadening effect and in acquiring vicariously a continuing desire for learning.

There should be no misunderstanding to the effect that a content of knowledge is considered unimportant, for that is not the case. It would be absurd to suppose that any process of medical education should not result in an effective content of knowledge, but this may be obtained by a flank rather than by a frontal attack.

It is necessary as a first consideration to decide what an effective content of knowledge may be for the student in his various stages of progress through the school. An approximation to this which is largely automatic obtains in the clinical years. Here, if the teaching is done by an intimate contact with the patient as a problem, the scope of the experience is determined by the frequency of various forms of disease in the clinic, which approximates that which the physician will later encounter. This is the central working focus, around which will accumulate his further experience and this should serve for learning habits of work, for acquiring skill in the use of the scientific method, and for becoming familiar with the technique of exploring the experience of others, that is to say, the use of medical literature. To make this process effectual, direct contact with the patient involving an appropriate degree of responsibility is more essential than the number of patients cared for, though this should be as large as the student can assimilate. It is at once granted that this does not provide a complete and systematic survey of the fields of clinical medicine or in fact of any

particular field. Such is not and cannot be the function of the medical school in its undergraduate years, but rather that of the graduate training where concentration is possible in special activities. The fact should be squarely faced that four years is not sufficient for acquiring a comprehensive and detailed content of knowledge of clinical medicine in all its divisions, and of its basic sciences. Emphasis must be placed upon the acquiring of habits of work and the common method for solving the problems involved; the development of a scientific attitude rather than of a scholastic one.

This does not mean that the student must necessarily be unaware of knowledge outside his direct experience. Means should be employed to see that he undertakes, himself, in his own time and under his own initiative a survey of the various fields, sufficient to make him aware at least of the scope of the problems with which he may be faced. Were there no literature available this would have to be done by the lecturer, as was necessary before the introduction of movable type. Today, with adequate texts, monographs, and current literature the lecturer is put to it to say anything that is not better said on the printed page. The lecture should be limited to work not yet so available, to original and sound points of view not otherwise obtainable, and to inspirational values peculiar to the lecturer, not in his estimation but in that of the student. Under these limitations lectures will be relatively rare, and never obligatory, and the inadequate ones will fall of their own weight.

The more intensive knowledge is better obtained in small groups of the seminar type presided over by a member of the faculty functioning as a commentator, but in which the student does the work. For this, time in which such groups may function must be set aside in the curriculum as elective undertakings, in contrast to the common basic requirements. Approximately one-third of the curriculum hours should be so disposed.

While this discussion has been undertaken from a clinician's viewpoint, it is equally pertinent to the basic sciences in the school of medicine. One cannot make an anatomist, a physiologist, a pharmacologist, a biological chemist, or a pathologist out of the student in the all too short two years available for these studies. But the student can become sufficiently familiar with such fields of study so that a content of knowledge will be available for reference in the future, and above all he can acquire an insight into the method of approach to the solution of problems in these fields. Again, this must be obtained by direct experience in the laboratories, by literally handling the problems and not by lecture and demonstration except within the limitations previously discussed. Here, also, the literature is adequate in

concise or in expansive form, as may be preferred, and the student should be led into the use of this himself, rather than by having it eructated by the teacher, whose ruminative assistance will not be available for him in his future professional life.

It will be asked, if the student be left to his own initiative for the acquisition of an adequate content of knowledge, can one be certain that he will do this? In the first place, it should be understood by faculty and student alike, that this is the student's responsibility. The faculty provides adequate opportunities, sympathetically advises, and outlines the paths along which the student must go, but *he* does the walking. It is no function of the faculty to provide him with transportation. The tuition is not carfare, but an entrance fee which covers only a small part of the costs of the caravan. It is to the student's interest primarily, rather than to that of the teacher, that he obtain an adequate medical education, and he should be disillusioned if he supposes that by the deposit of a fee he can transfer the weight of the burden to the faculty.

It is the obligation of the latter, however, to see that these opportunities, costly as they are, are not wasted on the incapable or the unwilling. The correction of such is not a matter of abstruse mathematics centering in the Dean's office, but of proper admitting to the school in the first place and, in the second, of weeding out the obvious defectives early in the journey.

Another query may be, "Is there no place for examinations in this process?" If the purpose of such is disciplinary and for direct control of the students' activities, the answer is, "There is *no* place for such." Once embarked on his professional life the student will not have an examining board to govern his activities. On the other hand, the examination may be made, if properly conceived and given, an educational adjunct of value. It is of considerable importance that the student have the experience of assembling and correlating his knowledge and putting it down in writing, but for this the questions should be of a comprehensive character rather than mere factual queries. This is of particular educational value at the end of the basic science years and again at the end of the clinical years, if time is left for review and thought in preparation for the examination. Again the important thing is not the ascertaining of a student's absolute or relative standing, but rather the subjecting of him to a valuable experience.

This discussion has been centered about the ideal "general practitioner" in order that there may be a starting point upon which all will agree. At the conclusion of it, however, it should be apparent that what has been said as regards desirable objectives in his education and the methods of attaining them is in every respect applicable to the specialist in every field

of clinical medicine, and to the worker in the field of the basic sciences. In particular, it should be emphasized that the old and still current concept of the necessity for a differing medical education for the prospective clinician and for the future research worker is a bugaboo which this discussion should have done something to lay low.

Education in general, and medical education is no exception, is, as one has said, "a continuing process of growth" which should not cease with the obtaining of a degree. It is a sensitive process, for the most part autocatalytic, which responds favorably to a proper environment. The most important objective of medical education is to see that such growth remains a continuing process throughout the future professional life of the student.

REPORT ON CURRICULUM *

Number of students: The number of students in the School of Medicine is determined by the number of the faculty and by the physical facilities. Inasmuch as the emphasis in teaching should be laid upon direct experience in the laboratory and the clinic, the capacity of the former and the patients available in the latter constitute the limitations of these facilities. Over a number of years it has been learned that a desirable maximum in the two clinical years is approximately 100 students, and the development of teaching personnel has been adjusted to this number. There is no reason for changing this number, and while it is not absolute, the number of students in each of these years should be 48 ± 4. In the basic science years the laboratory accommodations have been adjusted to a number approximately 10 per cent higher than this, thus allowing for a certain depletion before reaching the clinical years. This loss per class should be averaged for the past ten years and should be used to determine the maximum number of students admitted, as follows: $48 \pm 4 + x$.

Any substantial decrease in number would be regrettable, not only from the resultant loss of income but, of more importance, from an incomplete use of the present facilities. Any material increase without a proportionate increment in faculty and physical facilities would lead to overloading and mass-teaching methods, as by lecture, with a deplorable lowering of the standards of instruction.

Admissions: The determination of the number of admissions is discussed in the preceding paragraphs. The quality of those admitted is of the greatest importance, for this determines the quality of the men graduated which, in turn, determines to a considerable degree the reputation of the school.

Men of high quality will be attracted by a faculty of high ability and reputation and by the opportunities to learn by direct experience in the laboratories and the clinics. It is therefore necessary to seize every opportunity for improving the personnel of the faculty and the methods of providing instruction. In the latter respect the school has a unique opportunity, for the great majority of medical schools are teaching for the most part by methods which are antiquated and inadequate. The opportunity to lead in this respect is immediately at hand and should be grasped at once.

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* Dr. Harvey was at this time Chairman of the Committee on Clinical Subjects of the Yale School of Medicine and a member of the Committee on Clinical Research and Teaching.

Admission to the school should be based on an evaluation of the applicant's qualities as demonstrated by past performance, not on prophecy as to what he may or may not do in the school or thereafter. Integrity of character stands first, and is, at best, difficult to determine by any method aside from that of being closely associated with the candidate over a long time. Since that is usually impossible, such information may be gained from former associates, in which case it is reliable only if the informer is dependable and the communication personal and confidential. Something can be deduced from a knowledge of the candidate's social and family environment, though it must be kept in mind that economic and social standing by no means signifies integrity, nor does such a lack prove to the contrary. Personal interviews, as at present carried out, should be heavily weighted, but the interviewers must be carefully chosen for shrewd and sound judgment.

Intellectual capacity is of nearly equal importance, and this must be judged by past performance, by psychological testing, and by the interviewer's appraisal, as is being done at present. Any hope that low intellectual capacity can be increased by education should be abandoned. However, it should be kept in mind that an occasional candidate's past performance may be poor, not because of low intellectual capacity but as a result of failure to work or because of faulty habits of study, which may be rectified in the future experience. However, this is on the whole an unsafe hypothesis, and should not be too heavily relied upon.

A well-balanced individual is usually one of good judgment and therefore, other things being equal, is desirable. On the other hand, this standard may be overused, leading to a uniform type, and ruling out the occasional eccentric who may be a man of unusual and desirable potentialities.

It is also of importance to know the objectives of the applicant in studying medicine, frequently a difficult thing to ascertain. Any serious suspicion that the *primary* motivation is that of financial or social gain should disqualify him. However, it is necessary to guard against the assumption that, because a student comes from a low economic and social level, such is his motivation, and to bear in mind that many of the most able men in medicine have had such a derivation.

There should be, of course, no blanket discrimination as regards sex, creed, or race; on the other hand, it is equally unfair to have an unbalanced distribution as regards these categories, for this involves discrimination also in that it excludes individuals who would otherwise be acceptable. It is safe to say that a proper proportion of the various elements of the population represented within the student body should within wide limits correlate with that of the population of the country as a whole. The policy followed by the school in the past seems a sound one.

The faculty and teaching: It is a function of the faculty as a whole to enunciate general policies as to distribution of time and methods of teaching, while it is the obligation of the individual member to accommodate himself to these in so far as his conduct affects other members of the faculty. It is recognized that a teacher can best teach only when he does so in the way he thinks to be the best. This prerogative should be valued highly and so used.

The distribution of teaching as regards allotment of time and of fields of activity must of necessity, in the main, follow the traditional divisions. This is particularly true in the required or basic experience offered the student; in the elective opportunities the instructor has a greater chance for diversity and may follow his investigative interests into fields traditionally foreign to that of his departmental assignment.

Basic experience: There is in every field a certain basic experience, which is common to all activities in medicine, and in this the student should be soundly founded. Were it possible it might be desirable for every physician to be an anatomist, a physiologist, a surgeon, a psychiatrist, *et cetera,* but this is very obviously not feasible, even were the available time increased without limit, rather than being sharply delimited by four years. Nor does it serve the purpose to overload the working capacity of the student or to resort to forced feeding with the concept that a content of knowledge is the thing. Such efforts are ineffectual in the end and serve only to stunt educational growth.

It seems best to decide at first upon the proper number of basic and elective hours of which the student may be expected to take advantage. This must be done with knowledge that the student must also be left ample time for study, quite apart from scheduled exercises, for fatigue in intellectual effort is no less damaging to efficiency than it is in physical exertion. Thirty-six hours a week for 33 weeks (the scheduled school year) approximates 1,200 hours, which would seem a maximum and is very likely too much rather than too little.

Elective experience: The distribution between basic and elective hours must be determined, but it is first necessary to keep in mind the distinction between the two. The basic experience has already been defined, and it is at once apparent that even if all the time were used for this, the student would be left, from the standpoint of content, with a relatively superficial and elementary approach to medicine; he would never have had the experience of sinking his teeth deeply into any one subject. Moreover, the scientific method is best learned in an intensive approach. This means using a part of the time in small fields of interest with a vertical rather than a horizontal coverage. It is best carried out by relatively small groups of students (not over 12) working intimately and intensively with instructor and material in a restricted field. The success of the electives in previous experience has shown their value but the time allotted has in practice been seriously encroached upon. A proper proportion would be two basic units to one elective, or one-third of the time for the latter. The time distribution would then be in a given year:

Basic experience	800 hours
Elective experience	400 hours
Total	1200 hours

The basic experience should not be expanded by leaving out of it essential divisions which are subsequently presented as large group electives. The end result of such manipulation is to increase basic hours at the cost of elective hours, the appearance only of distribution having been maintained. This can be guarded against by limitation of the number of students allowed to take any given elective.

Aside from small-group electives there are certain other educational opportunities, some of which have been scheduled in such a fashion as to suggest that they are required exercises, though attendance has not been taken and in practice they are entirely elective. Such are certain lectures, clinics, and clinical-pathological conferences, and there are many other still more informal opportunities which fall into no definite categories. They are, in fact, admirable, provided there is no compulsion or no suggestion, direct or indirect, of such. They should not be used as a method of

expanding basic hours, nor should blocks of information be deleted from the latter in the expectation that these exercises will fill the purpose. All such should be listed in the catalog as electives which all students may attend when and as they choose. Their educational value, as compared with the direct experience of the basic courses, is little; for the most part they serve to arouse and maintain interest. One advantage of making them completely elective is that when they no longer compete successfully with conflicting opportunities they will fall of their own weight. Those surviving will have been proved to be of value.

Distribution of fields of experience: The distribution of fields of basic experience, according to the traditional departmental and divisional arrangement in the school, is adequate as is the sequence of assignment to these. This consists of anatomy, physiology, and biological chemistry in the first year; of pathology, bacteriology, pharmacology, and certain propaedeutic courses in clinical medicine in the second year; and in the third and fourth years the various divisions of clinical medicine and public health, to be considered more in detail later.

FIRST YEAR

In the first year anatomy has, by tradition, taken the dominant rôle. It was the first of the basic sciences to be taught in the laboratory at a time when precise knowledge was for the most part confined to morphology. This relative position has of course changed and today the functional aspects of the organism have assumed a constantly increasing importance. In many instances these can be better experienced by observation of physical and chemical phenomena susceptible to an experimental approach than as a part of the study of morphology alone. This should not be regarded as a degradation of anatomy but rather as a relative change in respect to the distribution of the student's time. It has, of course, nothing to do with the importance or character of the investigative work done in a department or of the elective time that it may see fit to offer.

A fair adjustment at present would seem to be the assignment to anatomy of 50 per cent of the basic time available, or 400 hours, and to physiology and biological chemistry 25 per cent each, or 200 hours. This is accomplished by utilizing the morning hours from 8 to 12, for 6 mornings a week for the 33 weeks of the school year, one-half of these being assigned to anatomy and one-half to physiology and physiological chemistry.

It should be observed that by doing this blocks of time for the basic divisions are set up in the mornings and that the afternoons are free for elective work. Cataloged scheduling within these blocks is unnecessary, for the arrangement of time, the fields covered, and the types of experience provided are an intradepartmental function to be arranged according to its best judgment. Courses that are offered in the afternoon hours must be entirely of an elective nature and not intended to continue the basic work of the morning. If, however, the student wishes to continue his work in the laboratories into the afternoon, and it is possible to do this, he should be able to do so but under no compulsion, direct or indirect, from the faculty.

However, the noon hour of 12 to 1, and the hours after 4:30 in the afternoon should be left free for the providing of opportunities of the "general elective" type as discussed previously. While not compulsory, some of these will be of sufficient importance so that the majority of the students will wish to attend them at one time

or another. Therefore, they should be open to all students, irrespective of chronological status in the school. This provides an opportunity for the students of the first two years to get some contact with the problems of the clinic, and for those of the clinical years to maintain a contact with the fundamental sciences throughout their experience in the school.

SECOND YEAR

The second year is essentially one of the study of the abnormal, and pathology occupies in it a position corresponding to that of anatomy in the first year. The other subjects involved are bacteriology and immunology, and pharmacology. The essential changes involved are the deletion of the scheduled courses in clinical medicine in this year, and the provision of adequate time for pharmacology and for electives.

The reasons for this change are several. In the first place all the time available in these two years is necessary for the basic sciences of medicine, and particularly so if a proper amount of time is reserved for the elective experience so essential to the proper education of the student. Pathology covers a wide field in abnormal morphology and physiology, while bacteriology deals with living causative agents of disease and the reactions resulting therefrom, and pharmacology with the action of non-living agents, a field that is rapidly changing and assuming new importance.

Certain courses in preparation for the clinical years have been given in the latter half of the second year. These are "Physical Diagnosis," "Clinical Microscopy," and "Introduction to Psychiatry." The teaching of these has been for the most part without contact with the patient on the one hand, and without integration with the related basic field in the preclinical years on the other hand. It would seem wise to bring these into close correlation with the fundamental sciences and with the clinical work; and this can be best brought about by breaking up the courses as given at present, and transferring appropriate sections to pertinent fields with the clinical faculty working with the preclinical faculty in obtaining the proper integration. It is not to be inferred that this is done with the intention of minimizing these subjects, but rather with the thought that they can be better learned in closer correlation with the fields of which they are a part.

THIRD AND FOURTH YEARS

The third and fourth, or clinical, years should be considered as one unit. The allotments of time are conditioned by the fact that the wards and clinics are the laboratories and patients are the subjects. These facilities are available in limited amounts but throughout the year, so that the student body must be broken up into groups which are manageable under these conditions. Therefore, the details of the arrangements must be quite different from those of the first two years, but the general principles underlying the experiences to which the student is exposed are the same.

This group arrangment has the advantage of offering the possibility of utilizing the material and plant for the purposes of learning throughout the year, and thus escaping the traditional attitude of the great majority of educational institutions that their facilities must lie idle for a third of the time. This is particularly appropriate where teaching is, for the most part, a process of learning by experience, and where the faculty by necessity of caring for patients must provide coverage throughout the year.

In order to take advantage of this, it is proposed that the students of the third and fourth years be divided among 8 groups, and that 8 periods of 11 weeks each, of which 3 are elective, be scheduled. This involves the use of the summer months for obligatory periods between the second and third years and between the third and fourth years. From the standpoint of the faculty, some additional attention would have to be paid to the students by those carrying the clinical services during this time, but this would entail no considerable burden.

Some concession would have to be made to those students who now earn part of their way during the summer period, although in principle this is undesirable. It would mean the choice by these of a group in which the elective time fell in the summer period. The assignment to groups should be in the first instance by choice, but it might prove necessary in some cases to give second or third choices. This would not involve any considerable hardship. The sequential progress from one division to another now obtaining could be maintained, although it is not of great importance. As for elective time, it would be provided in adequate blocks of completely clear time rather than piecemeal and in too little amounts as at present.

The one field that does not fit into this arrangement is that of public health, which at present is scattered throughout the week. It is suggested that in the fourth year this subject be given Wednesday mornings throughout the year, increasing thereby the time available and permitting the arrangement of its functions, within this block of time, as seems best to the department. This would involve the decrease of the Out-Patient Department time for medicine, pediatrics, urology, and orthopedics by one-sixth, but would retrieve for general use time now scattered in the afternoons of several days each week.

Electives: With the relegation of some educational opportunities now scheduled as required to the elective time and the provision of one-third of the total school time for electives, classification of the purpose and types of these is indicated. They will fall into three groups: (1) the seminar type, (2) the general conference type, and (3) the project type.

The *seminar type* does not differ from that which is being offered at the present time. At its best it provides an opportunity for the student to acquire additional experience in a concentrated field, either directly or by exploration of the literature, preferably both. If these are large groups, greater than 12 students for instance, or if the instructor is the active exponent and the student the passive recipient, the purpose is largely defeated. In general, the larger the number of electives offered the better, in order that there may be a greater diversity of selection. On the other hand, there should be no obligation in the number or character elected by the student. The list offered should be periodically reviewed and those not taken by a significant number of students over a period of time should be dropped from the catalogue. Likewise, new electives should be submitted for approval. This might well be the function of a special committee of the faculty as well as that of suggesting new electives when and where there seems to be a need for such.

The *general conference type* of elective includes a group of important educational opportunities which are conducted on the conference level. The conference phase here may well be between various members of the faculty. The Clinical Pathological Conference, the Neurological Study Unit, the so-called Grand Rounds in Surgery, and the noon-day clinics are examples of this. Where such degenerate into lectures their

potential value is for the most part inspirational, and it should always be remembered that but few members of the faculty have a facility of this nature. In no case should the student be given the concept that such conferences are systematic and offer an all-inclusive coverage of a basic field or are in any sense obligatory. On the other hand, such general conferences should, if physical facilities permit, be open to the student body as a whole. In this way, the student in the preclinical years may at his own discretion get a feeling of the significance of the fundamental sciences in clinical medicine.

The *project type* is at the present time exemplified by the thesis. This is another educational opportunity but, like the examinations to be discussed later, it is an opportunity for the student to organize a field of knowledge in a comprehensive manner. It differs from the seminar and general conference types in that this field is a vertical rather than a horizontal one, intensive rather than extensive. As such it is a legitimate requirement for graduation and should be used to a greater degree than at present in the evaluation of the student.

The opportunity for doing work of the project type should by no means be confined to the thesis. The investigation of various problems and the study of advanced techniques should be encouraged in every department of the school. In no other way can the use of the scientific method be better taught, and this is the major objective which should run throughout the instructional facilities offered to the student.

Examinations: The function of the examination is too commonly supposed to be that of determining the content of knowledge which the student contains at the moment, with the purpose of passing him if adequate and of failing him if inadequate. When given in course and at frequent intervals, it becomes a disciplinary measure for ensuring that the student does from day to day his job of absorbing information. It may be then an interesting game played with the faculty, in which the objectives are to mislead the latter into overestimation of the contestant's knowledge, and to acquire just the adequate amount for passing and no more. The primary objective becomes the passing of the examination, not the acquiring of an educational experience. As long, however, as degrees are a necessity and licensure obligatory, the examination remains, faulty as it is, the only possible way of ascertaining accomplishment. It can, however, be made an educational asset when properly conceived and given, and it is as such that the process should be critically examined.

The examination should be of the comprehensive type, that is to say, it should cover not courses or limited fields of experience, measured either by content or by time, but rather should be so comprehensive as to enforce the correlative and systematic review of these as an educational experience, difficult to provide otherwise. From this viewpoint the examinations as given at the end of the preclinical and the clinical years are satisfactory.

The examination should be given without reference to the teaching, and the teaching should be carried out without reference to the examination. Teaching should be directed at providing an educational experience not at preparing a student for an examination, and "refresher courses" have no place in the curriculum. The examination should be likewise directed primarily at being an educational experience and not for the purpose of passing or failing students. If both teaching and examining are so conceived there will be no serious lack of correlation, and the detection of superior or inferior students will be only incidental to the process.

The mechanics of providing an examination governed by these principles are not important. The written examination, using broad questions requiring integrative correlation rather than factual memory, is sufficient to innervate the educational objective. The actual questions might well originate in the various faculties concerned in the fields covered, but they should be assembled, correlated, and integrated by a "rewrite committee" which would provide the final version. The evaluation of the papers should be carried out as at present; perhaps the whole matter could be best handled through a "committee of the school," with subcommittees for the two examinations.

Again it should be emphasized that the objective of the examination is not that of discovery of inadequate or exceptional students. For this it should not be necessary, for these should be sufficiently conspicuous in the daily contacts of the students with the faculty. The inadequate student should not be admitted, but if admitted, should be detected early in his school experience and discarded. If he eludes these checks, he should be discovered incidentally in the first comprehensive examination and not allowed to continue.

With the educational experience of the student based upon the principles herein presented and guided by a curriculum such as that which has been suggested, there is reason to hope that the graduating student will be better equipped to assume his responsibilities to medicine.

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THE RÔLE OF ALCOHOL IN THE PATHOGENESIS OF CIRRHOSIS*

For over a century clinicians and pathologists have debated the rôle of alcohol in the pathogenesis of cirrhosis. Only in the last decade, however, has general agreement been reached on certain principles underlying the complex inter-relationships between alcohol ingestion, food intake, and alterations in the structure of the liver. Unfortunately, this has led to the erroneous impression in some quarters that the problem has been solved, and that further investigation is no longer needed. It is the purpose of this discussion, therefore, to review very briefly the evidence on which our current concept of the rôle of alcohol in the pathogenesis of cirrhosis is based and to call attention to some gaps in our knowledge.

In tracing the evolution of this concept it is possible to discern three distinct, but broadly overlapping, periods dominated in turn by the morphologist, the toxicologist, and the nutritionist. What I choose to call the morphologist's era may be said to have begun with Laennec's description[1] of the anatomical lesion in cirrhosis in 1826. It was a period marked by a bitter controversy between the proponents of the view that alcohol was the direct cause of cirrhosis and those who denied any relationship between the two. In the end, however, both sides were forced into the intermediate position so well summarized by Jolliffe and Jellinek[2] in 1941:

> Even the most valid statistics, vital or clinical, cannot go beyond the point of indicating the association or non-association between cirrhosis of the liver and inebriety. These investigations in no way answer the question whether or not there is direct causation of cirrhosis of the liver from alcohol. Even statistics of 100 per cent validity showing the association could not be interpreted in terms that cirrhosis is caused by alcohol. The statistics merely indicate that among chronic alcoholics the occurrence of, as well as the death from, liver cirrhosis is significantly greater than among temperate persons.

It is of interest to note that these views were anticipated by Matthew Baillie[3] in his treatise on morbid anatomy published in 1793:

> This disease is hardly ever met with in a very young person, but frequently takes place in persons of middle or advanced age; it is likewise

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more common in men than women. This would seem to depend upon the habit of drinking being more common in the one sex than in the other; for this disease is most frequently found in hard drinkers, although we cannot see any necessary connection between that mode of life and this particular disease of the liver. It happens, however, very commonly, that we can see little connection between cause and effect in changes which are going on in every other part of the body.

The similarity of this remarkable statement to that made 146 years later by Jolliffe and Jellinek[2] must not be construed as evidence that no progress was made during this period. Actually three important contributions were made, without which further progress would have been impossible. First, the clinical and pathological features of Laennec's cirrhosis were clearly defined; second, it was shown that the disease occurred not only in association with chronic alcoholism, but also under a variety of other conditions; and finally, it was clearly established that a number of unrelated hepatic lesions were capable of producing different types of cirrhosis but that these could not always be distinguished from one another morphologically.

It is not surprising, considering the striking pharmacological actions of alcohol, that the toxicologist soon took up this problem. Starting in 1852 with Dahlström,[4] one investigator after another attempted to reproduce Laennec's cirrhosis in experimental animals by feeding large quantities of alcohol. These efforts almost uniformly met with failure,[5] so that the theory of a direct toxic action of alcohol in the liver had to be abandoned. However, the observation that alcohol enhanced the hepatotoxic effects of other agents, such as carbon tetrachloride,[6] chloroform,[7] and phosphorus,[8] led to a revival of the theory in a somewhat modified form, as exemplified in the following excerpt from Virgil Moon's review[5] on experimental cirrhosis:

> Without minimizing the contributory or predisposing influence which alcohol may exert, it must be concluded that experimental evidence has not substantiated the belief that alcohol is a direct cause for cirrhosis. . . . It is probable that such influence as alcohol exerts in causing human cirrhosis results from its action in reinforcing or accentuating the effects of other agents or in producing degenerative changes in the hepatic cells, thereby rendering them more susceptible to injury.

Although toxicological studies failed to define the rôle of alcohol in the pathogenesis of cirrhosis any more precisely than indicated by this rather vague and inconclusive statement, they did serve the useful purpose of introducing the new concept that alcohol might exert its effects on the liver in some indirect manner. At first this led to an unsuccessful search for toxic contaminants in alcoholic beverages such as phosphorus[9] and copper[10]; then, to the theory that the gastro-intestinal disturbances initiated by chronic

alcoholism facilitated the absorption of toxic substances[11]; and finally, to the current hypothesis that cirrhosis is the consequence of the nutritional deficiency commonly found in heavy drinkers.

Another important development during this period was the demonstration that alcohol could be utilized as a food under certain conditions. This had been suspected for some time but had not been previously investigated.

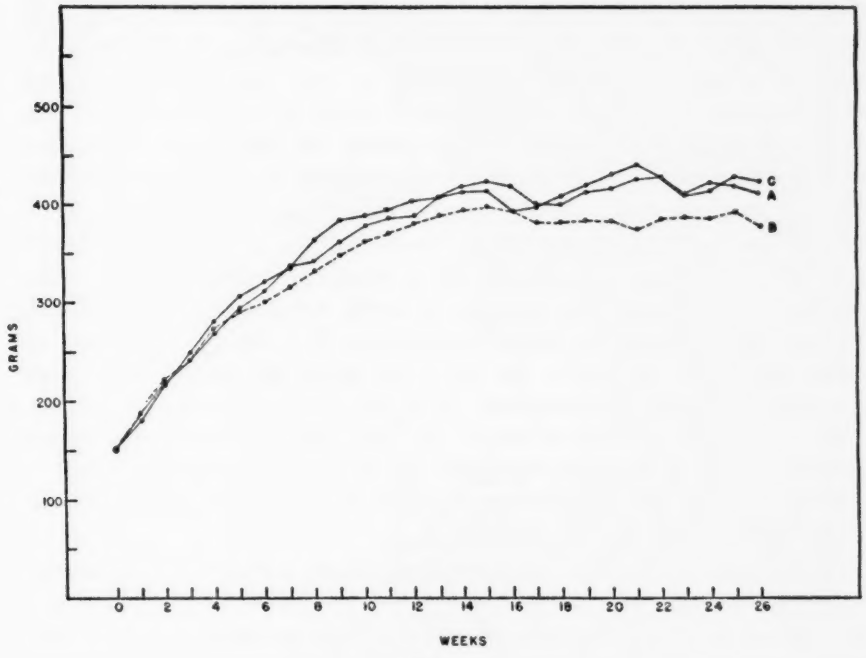

FIG. 1.

The results of these studies clearly showed that alcohol had a caloric value of approximately seven calories per gram and that it was capable of supporting growth,[12] although not to the same extent as isocaloric equivalents of sucrose when conditions were adequately controlled.[13] The effectiveness of alcohol in supporting growth is well illustrated in Figure 1 which depicts the growth curves of three groups of pair-fed rats studied in our own laboratory.[14] The food intake was identical in all three groups, but Group A received a 15 per cent solution of alcohol in lieu of drinking water, and Group C was given a supplement of glucose calculated to be isocaloric with the amount of alcohol drunk in Group A. It can be seen that both supplemented groups attained a significantly greater weight at the end of six months than their pair-fed controls in Group B, suggesting that alcohol

was about as effective as an isocaloric glucose supplement in supporting growth—at least to the limited extent demonstrated in this experiment.

Just how the energy of alcohol is utilized is not known precisely. Since there is no increase in total heat production during its oxidation, it is reasonably certain that an increase in specific dynamic action cannot account for the energy expended.[15] There is also good evidence that alcohol is rapidly and completely oxidized to carbon dioxide[16] irrespective of the amount ingested and that none is stored or converted to carbohydrate or fat.[15] Therefore, since alcohol is capable of supporting growth to some extent,[12, 13] it must be assumed that it spares carbohydrate and fat in certain energy reactions. However, there is considerable doubt that it can furnish energy for muscular contraction.[15] It is difficult to understand why alcohol should not be utilized as well as other foodstuffs in all energy reactions when one considers the pathways of its oxidation. As far as is known, alcohol undergoes progressive oxidation to acetaldehyde and acetic acid in the liver and thence to carbon dioxide in the tissues generally.[16, 17] From what is known about the fate of acetate in the body, it might be anticipated that the fraction derived from alcohol could be utilized in a wide variety of energy reactions. However, the apparent failure of alcohol to provide energy for muscular contraction would appear to be an exception. Recently it has been suggested that the reason for this is not that the energy cannot be utilized, but rather that the relatively fixed rate at which alcohol is oxidized to acetic acid in the liver limits its availability in response to the sudden demands of muscular activity.[15]

Alcohol also differs from other foodstuffs in its nitrogen-sparing action. Although alcohol supplements increase the storage of protein in young growing animals receiving a liberal diet, they fail to spare nitrogen in animals on low-protein diets.[15] The reason for this difference is still not known, but may be related to the previously mentioned limited capacity of alcohol to participate in certain energy reactions. Although these metabolic aspects of the alcohol problem have thus far received little attention in studies concerned with the pathogenesis of cirrhosis, it is highly probable that they will be the center of interest in future investigations.

Our current concept of the relationship between alcohol and cirrhosis is based on the premise that the chronic ingestion of alcohol leads to, or is associated with, a specific type of dietary deficiency which results in fatty infiltration of the liver, and that the development of cirrhosis is the direct consequence or sequel of such infiltration. In short, alcohol is relegated to a purely secondary rôle in the pathogenesis of the cirrhotic lesion.

In tracing the origins of this concept we find no direct chain of evidence but rather a series of isolated and apparently unrelated observations made

over a period of years by pathologists, clinicians, physiologists, and chemists. Only in the past fifteen years have these been integrated and extended by a group of active investigators primarily interested in nutrition. It is for that reason that I have designated this the nutritionist's era.

Perhaps the first reference to the association between fatty liver and alcohol is to be found in a statement made by the English clinician, Thomas Addison[18] in 1836:

> With respect to the causes of this fatty degeneration of the liver, very little, or absolutely nothing, is known. In most of the cases which I have met with, there has been either positive or strong presumptive evidence that the individuals had indulged in spirit-drinking; and indeed the most exquisite case I ever saw in a young subject, occurred in a female who had for some time subsisted almost exclusively on ardent spirits.

This observation was amply confirmed by others although it was appreciated from the beginning that alcohol was not the only cause of fatty liver. It was not until 1849, however, that the Viennese pathologist, Rokitansky,[19] first suggested that fatty infiltration might be the precursor of cirrhosis. Some ninety years later Connor affirmed this view[20] and then provided experimental proof of the progression of fatty infiltration to cirrhosis both in depancreatized dogs maintained on insulin[21] and in normal dogs fed high-fat diets.[22]

At about the same time a group of Canadian physiologists discovered that a number of substances, which they termed lipotropes, could either prevent or cure the fatty infiltration which occurred in depancreatized dogs. Starting with raw pancreas, which had proved to be highly effective,[23] they soon found that one of its constituents, lecithin,[24] and its major component, choline,[25] were equally active. Subsequently it was shown that these agents also exerted a lipotropic effect on the fatty livers of animals maintained on high-fat diets.[26,27] These observations led to the hypothesis that an adequate supply of phospholipid, derived either directly from the diet or by synthesis *in vivo* from dietary choline, was essential for the normal transport of fat in and out of the liver.[28] However, it was soon found that certain choline-free, purified proteins, such as casein,[29] and at least one amino acid, namely methionine,[30] were also potent lipotropes. Fortunately, the physiologists were spared the necessity of revising their phospholipid-lipotrope theory by the timely discovery of a chemist, du Vigneaud,[31] that choline could be readily synthesized *in vivo* from ethanolamine, provided an adequate supply of labile methyl groups was available, and that methionine, or any methionine-containing protein, was a ready source of this material.

As a logical consequence of these discoveries, it was soon demonstrated that animals maintained for long periods on diets deficient in lipotropic sub-

stances developed a type of fatty cirrhosis closely resembling that seen in man,[32–35] and thus the missing link between alcoholism and cirrhosis appeared to have been found. However, even before the results of these experiments were widely known, it had been observed that alcoholics often exhibited signs of nutritional deficiency, and that dietary supplements were not only effective in restoring nutrition, but were also capable of improving the functional status of the liver when cirrhosis was present.[36] It was to be expected, therefore, that clinicians would seize on these interesting experimental observations and apply them to the problem of alcoholic cirrhosis. This led more or less directly to the hypothesis that Laennec's cirrhosis was the consequence of a dietary deficiency conditioned by over-indulgence in alcohol and to the widespread adoption of high-protein diets as the mainstay of therapy in this disease. Strong support for the hypothesis was not long in coming. First there were numerous reports[37–40] testifying to the therapeutic effectiveness of protein supplements, then the demonstration that alcohol did not impede recovery from cirrhosis, provided it was accompanied by a high protein intake,[38] and finally the recognition of dietary deficiency as an important factor in the etiology of the non-alcoholic endemic cirrhosis found in various parts of the world.[41–43] So impressive was this body of evidence that for a number of years no dissenting voices were heard, a remarkable state of affairs in the history of the alcoholic cirrhosis problem. Recently, however, clinicians have begun to take note of some of the inconsistencies in the theory, and several groups of investigators have undertaken to re-examine the problem experimentally. The following are a few of the clinical observations which have given rise to these doubts:

(i) Not all alcoholics with cirrhosis exhibit signs of malnutrition nor admit to deficiencies in their diets; in our own experience this has been particularly true of heavy wine and beer drinkers.[44]

(ii) Laennec's cirrhosis is uncommon in malnourished non-alcoholics with chronic debilitating diseases, such as tuberculosis, ulcerative colitis, and anorexia nervosa.[44]

(iii) Cirrhosis has not been observed in certain groups of individuals starved or underfed for long periods. Thus, in a study of 300 American soldiers recently returned from Japanese prison camps, there were no unequivocal instances of cirrhosis despite the fact that all had suffered from severe malnutrition for periods of two to three years.[45] The same was found to be the case in a group of malnourished Germans studied by Sherlock.[46] In this instance the evidence was all the more impressive since biopsy specimens of the liver were obtained to confirm the negative clinical and laboratory findings.

(iv) Improvement in the clinical, functional, and histological status of the liver has been observed in cirrhotics maintained on suboptimal diets.[47] Indeed, in short-term studies[48] similar improvement has been observed in subjects receiving diets practically devoid of protein.

Clinical observations such as these, while in no way discrediting the importance of nutritional factors in certain types of cirrhosis, have, nevertheless, raised the question once again of whether alcohol may not play a more direct rôle in the pathogenesis of Laennec's cirrhosis than by simply reducing the dietary intake. It is of interest in this connection to note that the investigators who have contributed most to our knowledge of experimental dietary cirrhosis have been far more conservative in interpreting their results than have clinicians. Certainly the very special conditions required for the production of dietary cirrhosis in animals are not duplicated in the alcoholic who develops cirrhosis. Moreover, certain morphological differences in the two conditions, and especially the distribution of the heptic fibrosis,[49] underscore the need for caution in translating the results of animal experiments in terms of human cirrhosis.

Despite the negative results obtained by others in the past,[5] a number of workers[14,50,51] have recently re-investigated the rôle of alcohol in experimental cirrhosis. In contrast to previous studies, due consideration has been given to dietary factors in these experiments. The first of these was carried out by Ashworth in 1947. He found that when large amounts of alcohol were administered to rats receiving a high-protein diet, they developed grossly fatty livers within a period of fifty days in contrast to pair-fed controls who did not. Since the intake of lipotropic substances was identical in the two groups, he concluded that alcohol exerted a direct effect on the liver which resulted in an accumulation of fat, and that this effect was not the consequence of an induced extrinsic deficiency of lipotropic factors. Unfortunately, the amounts of alcohol used were excessive and produced periods of coma resulting in a poor dietary intake and weight loss. The weight loss was particularly notable in the non-alcoholic pair-fed controls. Moreover, as is evident from Figure 2, the two groups of animals, while receiving the same amount of food, were not isocaloric, since no account was taken of alcohol calories. These were serious defects in the experiment since the presence of fat in the alcoholic group could have been due to the consumption of extra calories or, conversely, the absence of fat in the non-alcoholic rats could have been related to the more severe malnutrition that was present.

To obviate these difficulties Best and his associates[51] repeated the Ashworth experiment, substituting a 15 per cent solution of alcohol as the sole source of drinking water, and including a second control group which was not only pair-fed, but also isocaloric. The latter was accomplished by adding

a sucrose supplement. Thus, as indicated in Figure 2, the alcohol-fed animals (Group A) had two sets of pair-fed controls, one that was isocaloric (Group C), and one that was not (Group B). Under these conditions the animals exhibited no signs of alcohol intoxication and grew well. The diet, which was lower in protein content than Ashworth's, was designed to be marginal with respect to lipotropic activity, so that animals fed the diet *ad libitum* without alcohol (Group E, Fig. 2) had approximately 9 per cent

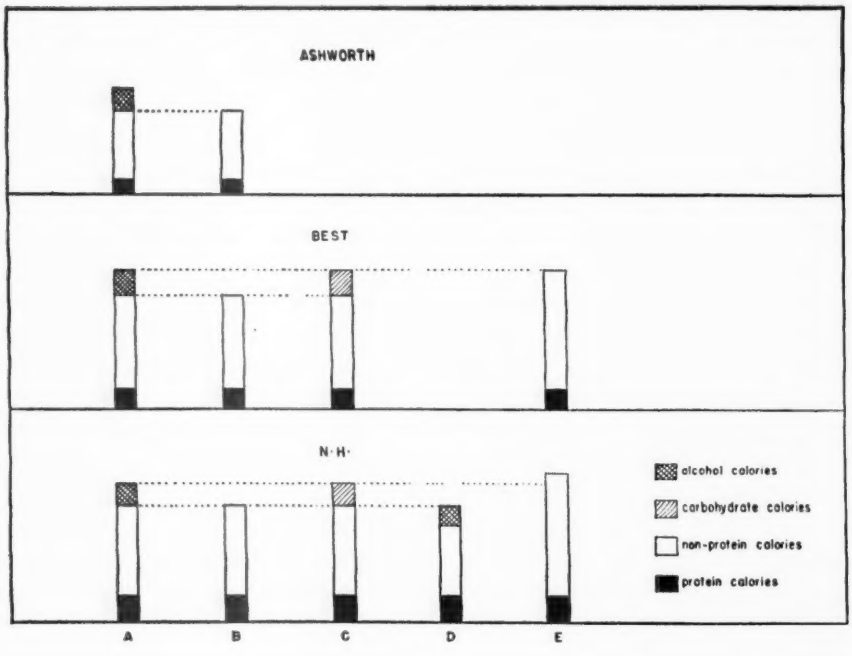

FIG. 2.

fat in their livers, a value twice normal. When the animals were sacrificed at 177 days, it was found that the alcohol-fed group (Group A) and their pair-fed isocaloric controls receiving a sucrose supplement (Group C) both showed a great increase in hepatic fat and what was termed a pre-cirrhotic fibrosis. Interestingly enough, the hepatic changes were more severe in the sucrose-supplemented group. On the other hand, fatty infiltration and fibrosis were not seen in Group B which was pair-fed but not isocaloric with Group A. It was also found that the hepatic changes seen in the alcohol- and sucrose-supplemented groups could be prevented by adding casein, methionine, or choline to the basal diet.

Since the effects of alcohol and an isocaloric equivalent of sucrose appeared to be identical, and since they could be prevented in either case by supplementing the basal diet with lipotropic substances, it was concluded that alcohol had induced a choline deficiency by augmenting the caloric intake. In short, it was implied that the choline requirement was a function of the caloric intake.

It must be pointed out, however, that while Best's results were consistent with this hypothesis, they by no means established it. In particular, it was not demonstrated that the effects of alcohol could be abolished by restricting calories, a proof that would appear to be essential. For that reason it seemed important to us[14,52] to repeat these experiments with an additional group of controls in which the caloric intake was not permitted to rise when alcohol was consumed. This was accomplished by subtracting isocaloric equivalents of carbohydrates. As a result, the animals designated as Group D in Figure 2 received the same amount of alcohol as in Group A but were kept isocaloric with the non-alcoholic rats in Group B. It should be emphasized that the amounts of protein consumed, and hence the available lipotropic factors, were identical in all four experimental groups.

If, as Best suggested, alcohol and sucrose increased the choline requirement by augmenting the caloric intake, it was to be anticipated that alcohol would have no such effect in Group D animals since their caloric intake was no greater than that of the pair-fed animals in Group B receiving neither alcohol nor sucrose supplements. Actually, however, both groups of alcohol-fed rats (A and D) showed larger amounts of fat and more fibrosis in their livers than the non-alcoholic rats in Group B, and, even more significantly, the hepatic lesions in Group D were more severe than in Group A, despite the fact that their caloric intake had not been permitted to rise when alcohol was consumed. It was concluded, therefore, that if alcohol increased the choline requirement, it did not do so by augmenting the caloric intake. The observation made by Best that an isocaloric sucrose supplement produced the same type of lesion was confirmed. It was also confirmed that supplements of choline and methionine, or an increase in the casein content of the basal diet, protected against the effects of both alcohol and sucrose. These observations were consistent with Best's hypothesis that both alcohol and sucrose increased the choline requirement. However, the data did not permit any conclusions regarding possible mechanisms underlying this phenomenon, nor did they warrant the assumption that the mechanisms were necessarily the same in both instances.

In comparing the results in Best's experiments with our own it was noted that, although his animals had a substantially greater intake of protein, and hence of lipotropic factors, they showed more severe hepatic lesions, sug-

gesting a more severe choline deficiency. The reason for this discrepancy is not clear. However, it should be noted that almost half of the protein in Best's basal diet consisted of gelatin and zein—poor quality proteins known to be deficient in several essential amino acids. This raises the question of whether specific protein deficiencies, unrelated to the synthesis and utilization of choline, may not play a rôle in the pathogenesis of alcoholic cirrhosis. Although this possibility was not investigated, at least one bit of evidence was uncovered which was suggestive. It was found that when the degree of fatty infiltration in our alcoholic rats was not sufficiently great to obscure its localization, it occurred more commonly in the periportal areas than in the central zones. Since choline deficiency characteristically leads to deposition of fat centrally, it is conceivable that the changes noted were due to some other cause.

Since casein must undergo digestion and absorption before its methionine component can be utilized in phospholipid synthesis, alcohol could conceivably have interfered with these processes by affecting the pancreas, which it appears to do not infrequently in man,[53] or by altering the absorptive membrane of the intestinal tract. For that reason the histology of the pancreas and the fecal excretion of nitrogen were investigated. No significant pancreatic lesions were observed, and the fecal excretion of nitrogen was found to be lower in both alcohol-fed groups than in their controls, an effect of alcohol previously noted by Mitchell.[13] Thus, there was no reason to believe that these effects were the consequence of impaired protein digestion or absorption.

The fact that the effects of alcohol could be abolished by supplements of casein, methionine, or choline suggested that there was no defect in choline synthesis or utilization. However, the amounts used were quite large so that a minor defect could have been masked. Moreover, there were a number of other theoretical ways in which alcohol might have exerted an unfavorable effect on these mechanisms which were not investigated. A few of these might be cited to indicate some of the areas that remain to be explored.

De la Huerga and Popper[54] have shown that more than half of an administered dose of choline is promptly broken down to trimethylamine and its oxide by the intestinal flora and then excreted in the urine. Thus, a large fraction of ingested choline is normally unavailable for lipotropic purposes. What effect alcohol has on this process is not known, but it could conceivably be an important factor in the pathogenesis of fatty liver if it increased such losses.

The labile methyl groups derived from methionine are by no means all available for choline synthesis, since many are diverted to other compounds requiring methylation. In at least one instance, the urinary excretion of

N'-methylnicotinamide, it has been shown that the losses of methyl groups are increased by alcohol ingestion.[55] Although the losses in this case would appear to be too small to account for the development of a choline deficiency, the possibility of more significant losses in other compounds has not been investigated.

Another way in which alcohol might increase the demand for choline is by interfering with the absorption or utilization of vitamin B_{12}, since there is convincing evidence that the choline requirement depends in part on the availability of vitamin B_{12}.

In addition to choline and its precursors, the normal synthesis of phospholipid requires a mixture of fatty acids. While endogenous fat can supply most of this demand, the body must depend on an exogenous supply for certain of the unsaturated fatty acids which it cannot synthesize. When these are not available, phospholipid synthesis is depressed and fat accumulates in the liver.[57] It is conceivable, therefore, that alcohol might induce an apparent choline deficiency by interfering with the absorption or utilization of such fatty acids.

These by no means constitute a complete inventory of all the possibilities, but they serve to illustrate some of the ways in which alcohol might produce a fatty cirrhosis without necessarily reducing the dietary intake of lipotropic factors or acting as a direct hepatotoxin.

Another aspect of the alcohol problem which is pertinent to our discussion is the effect of alcohol ingestion on food intake. There seems to be little doubt that the vast majority of heavy drinkers reduce their intake of food. This is generally attributed to the anorexia that accompanies alcoholic gastritis, to the mental apathy engendered by excessive drinking, and to the economic burden imposed by the high cost of alcoholic beverages. Although these factors do play a rôle, there appears to be a far more fundamental physiological basis for the decline in appetite—and that is the large number of calories ingested as alcohol. Some years ago Cowgill demonstrated the important principle that there is a normal homeostatic mechanism for the maintenance of a reasonably constant caloric intake. Thus, he was able to show that when a dog's diet was diluted calorically by adding some inert material, the dog simply ate more food, and in the end consumed the same number of calories. Similarly when the diet was concentrated, by extracting water or by substituting fat for carbohydrate, the dog merely reduced the amount of food it ate to maintain its caloric intake at the original level.

It was of interest to us, therefore, to see whether the ingestion of a large number of alcohol calories would reduce the consumption of food in experimental animals and whether the total calories would remain constant. As can be seen in Figure 3, such proved to be the case. It will be noted that

Group A rats that were obliged to drink 15 per cent alcohol instead of water for fluid consistently ate significantly less food than their non-alcoholic controls in Group E. However, when their total caloric intake, including alcohol calories, was calculated, it was found to be almost identical with that in Group E.

While this effect of alcohol calories on food consumption has not been established in man, there is every reason to believe that it exists. Considering the fact that a heavy drinker may ingest from 2000 to 2500 calories

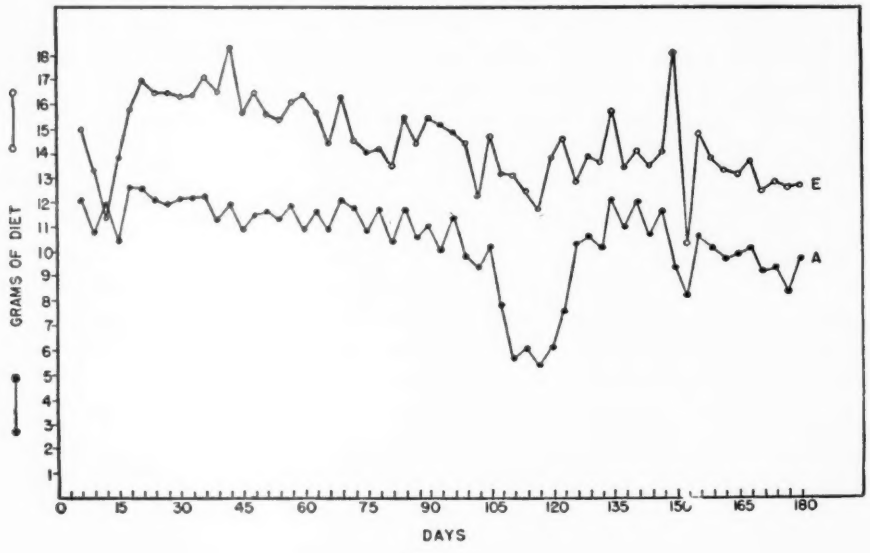

FIG. 3.

daily in the form of a quart of whiskey it is small wonder that he has no appetite for food. Nor is it surprising to find him looking well nourished, despite the fact that his food intake has dwindled markedly. Nevertheless, this maintenance of an adequate caloric intake is made at the expense of essential nutriments, and especially of proteins and vitamins, so that specific deficiency diseases may occur despite the maintenance of weight. Moreover, in the case of man, in contrast to the rat, long-continued drinking ultimately produces a *pathological* type of anorexia, which now reduces the caloric intake too. It is in these subjects that one finds overt signs of malnutrition and the most severe deficiency states.

In the light of these remarks, we must guard against going too far in transposing the results of the animal experiments described to the problem of Laennec's cirrhosis. No matter what rôle alcohol may ultimately be found to play in the pathogenesis of this disease, it must not be forgotten that,

even in the experimental animal, the diet must be at least marginal with respect to lipotropic factors before the effects of alcohol become evident, and that alcohol, by reducing the dietary intake, sets the stage, so to speak, for its own more specific deleterious effects in the liver. Thus, it will be recalled that animals given a high-protein diet showed none of the ill effects of prolonged ingestion exhibited by animals subsisting on a low-protein diet.

On the other hand, the effects of alcohol in the liver should not be dismissed as being purely secondary to a reduction in food intake even though it is recognized that protein deficiency itself can produce cirrhosis. Returning to our animal experiments, it will be recalled that, while the effects of alcohol were not evident unless the animals were on a low-protein diet, control animals receiving identical amounts of the same diet *without* alcohol showed no changes in their livers. Thus, it can be inferred that, in the experimental animal at least, alcohol probably has two effects—a nonspecific calorigenic effect which leads to a reduction in food intake and another which is more specifically related to the mechanisms for fat transport in the liver. The latter effect appears to be readily abolished by lipotropic agents which probably explains why it escaped recognition in animal experiments for so long. It may also explain the results of such human experiments, as Volwiler and Jones's[88] in which large amounts of alcohol failed to affect the liver adversely, provided a high-protein diet was maintained. However, it must be emphasized that this specific effect of alcohol has not yet been demonstrated in man. Unfortunately, at the present time, there is no way of demonstrating this effect except by the elaborate paired-feeding technique used in animal experiments, so that clinical investigation along these lines is not feasible. However, should the precise nature of alcohol's action be worked out in animals, it may be possible to demonstrate it in the human subject. Meanwhile, it can only be stated that many clinical and experimental observations are consistent with the hypothesis that alcohol has these same two effects in man.

In conclusion, a review of the evidence reveals that while dietary factors play an important rôle in the pathogenesis of alcoholic cirrhosis, a great deal remains to be learned about the complex interrelationships between alcohol consumption, dietary deficiencies, and alterations in the structure of the liver.

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SENSITIVITY TO COLD IN THE HEREDITARY OBESE-HYPERGLYCEMIC SYNDROME OF MICE*

The metabolic characteristics of a new Mendelian recessive syndrome in mice have been described in previous publications. The condition is characterized by an adult body weight of 50-100 g. instead of a normal 20-30 g., sterility,[10] hyperglycemia and glycosuria,[17] atrophic changes and ulceration of the skin, and decreased life span. Histopathological studies reveal enlarged islets of Langerhans; no definite histological evidence of other types of endocrine disturbances has been noted.[8] Total basal oxygen consumption is not greater than that of the non-obese animals in spite of a much greater body weight.[19] Spontaneous running activity is low.[15] Although obese animals show sensitivity to thyroxine as indicated by respiratory rate, weight loss, and toxicity, studies on radio-iodine uptake and of resistance to anoxia yield no evidence of impairment of thyroid function.[7] Administration of thiouracil to non-obese animals fails to reproduce any aspect of the syndrome.[20] The caloric intake of the obese mice is about 25 per cent greater than that of the non-obese. If allowed to select their nutrients freely, they choose more fat and less carbohydrate than their normal weight siblings.[18] Hyperglycemia develops at about the twelfth week of postnatal life. This diabetic-like condition is extremely resistant to insulin, is sensitive to growth hormone, and is dependent on the nature and amount of the diet. The obese animals are fatally affected by pancreatectomy and ACTH treatment.[20] They exhibit a partial block of acetate oxidation. By means of tracer techniques, it has been demonstrated in the mice that unoxidized acetate is converted for the main part into fatty acids, in smaller proportion into cholesterol.[8] It has been suggested that there is an etiological relationship between the partial block of acetate oxidation, obesity and hyperglycemia,[20] and between reduced glucose phosphorylation and hyperphagia.[14] The pri-

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mary block to acetate oxidation appears to sensitize the animals to the diabetogenic action of growth hormone. Reduced glucose utilization is, in the glucostatic concept of regulation of food intake,[14, 16, 25] responsible for increased appetite.

While the apparent extreme metabolic inertia of the adipose tissues of these animals can be overcome by fasting,[20] it appeared of interest to see whether in other conditions in which animals have to call on their reserves, the obese mice would be able to do so. Exposure to cold provided such conditions. It is known that, during exposure to cold, resting metabolism can be increased up to four or five times the basal rate.[5, 6] The effect of exercise was also examined, since increased muscular activity is an important component in the resistance to cold.[11] Because the physiological response to cold is mediated through the thyroid[12, 23] and the adrenals,[14] it appeared of interest to test the effect of thyroxine, thyrotrophic hormone, and ACTH on resistance to cold of obese animals as well as the effects of 2-4 dinitrophenol, a hyperthermic agent.[13] The histological effects of exposure to cold on the adrenals and thyroid were examined in both obese and non-obese animals.

METHODS

Young adult mice four to five months were used in these experiments. The weight of the obese animals was 45 to 55 g., that of the non-obese, 15 to 25 g. All animals had been raised in individual cages and fed Purina Laboratory Chow.

A total of 59 obese and 20 non-obese animals were used in the survival experiments. In addition, six obese and six non-obese mice, exposed to cold but not otherwise treated, were used for histological studies. The non-obese mice were killed at the same time the obese succumbed to the cold stress. Untreated obese and non-obese mice kept at 25° C. were sacrificed for histological studies.

The experimental animals were placed in bare, raised-bottomed cages in a cold room maintained at 3° C. Water and food were allowed *ad libitum* unless otherwise mentioned. Activity measurements of eight mice were conducted with the use of squirrel type cages with 32 cm. diameter wheels. Thyroxine, ACTH (Armour), thyrotropic hormone (Armour), and 2-4 dinitrophenol were administered daily by subcutaneous injections during the 48 hours preceding the day of exposure to cold. Dosages are given in Table 2. All animals exposed to cold were observed for survival every 20 minutes during the first two hours, every hour during the next six hours, approximately every two hours afterward until the end of the first day. The total time of survival was noted in each instance.

Adrenal and thyroid glands of the six unexposed obese and non-obese mice, of the untreated obese mice which succumbed in the cold and of their untreated non-obese controls (see above), were weighed and fixed in neutral 10 per cent formalin. Frozen sections at 15μ were made of the adrenals. They were (a) stained with sudan black B or sudan IV, (b) mounted in glycerine and examined with the polarizing microscope,

(c) stained by the Ashbel-Seligman method[2] for carbonyl compounds, or (d) extracted in absolute acetone for one hour at room temperature and treated as in (a) and (b).

The thyroid glands were dehydrated, embedded in paraffin, and sectioned at 5μ in the routine manner. After deparaffinization, the sections were stained with hemotoxylin and eosin and cell heights in the follicles determined according to a method previously described.[22]

RESULTS

Table 1 demonstrates the extreme resistance of non-obese mice to a prolonged cold stress. All exposed animals survived a seven-day exposure

TABLE 1. EFFECT OF SIMPLE EXPOSURE, FASTING, AND ACTIVITY ON THE DURATION OF SURVIVAL IN THE COLD OF HEREDITARILY OBESE-HYPERGLYCEMIC AND CONTROL MICE

	Non-obese mice			*Obese mice*		
Treatment	*None*	*Fasting*	*fasting*	*None*	*Fasting*	*fasting*
Number of animals	12	4	4	21	4	4
Duration of survival (Av. and range)	> 1 week	48 hrs.	15 hrs. (3-48)	2.2 hrs.* (0.5-5.5)	1.5 hrs.	3 hrs. (1-6)
Number of rev./hr.	.....	.....	266 (100-516)	.....	.....	98 (21-112)

* Out of the 21 animals, 19 died between one and three hours after the beginning of exposure.

period. Even when deprived of food, the non-obese mice were still resistant. All exposed animals survived the 48-hour exposure test period. It was only when a combination of fasting and increased activity was used that the limits of resistance to cold of the non-obese mice were overrun. By contrast, obese mice died in about two hours. Fasting and exercise brought about little difference in survival time in the obese mice.

Thyroxine, thyrotrophic hormone, and 2-4 dinitrophenol increased the duration of survival in the cold of exposed obese mice (Table 2). Graded doses of thyroxine had graded effects, but even a large, pharmacological dose of 100 μg. per day (approximately 2 mg./Kg./day) only prolonged survival by a few hours.

On the other hand, corticotrophin was rapidly fatal to the obese animals, definitely shortening their survival in the cold.

Histological examination of adrenals of obese and non-obese animals before exposure to cold showed only slight differences. The zona glomerulosa of non-obese animals contained usually less sudanophilic, birefringent, and Ashbel-Seligman-positive material than the corresponding zone in the obese animals. The lipid droplets of the zona fasciculata exhibited in both obese and non-obese specimens strong reactions to Sudan and the Ashbel-Seligman reagent. The birefringent pattern was somewhat less extensive. The lipid droplets were usually larger and less numerous in the fasciculata of obese mice. The presence of large lipid droplets in this zone is suggestive

TABLE 2: EFFECT OF VARIOUS TREATMENTS ON THE DURATION OF SURVIVAL IN THE COLD OF HEREDITARILY OBESE-HYPERGLYCEMIC MICE

(All treatments administered for 48 hours before exposure)

Treatment	*None*	*Thyroxine (25 μg./day)*	*Thyroxine (100 μg./day)*	*Thyrotrophic hormone (25 mg./day)*	*2-4 dinitrophenol (25 mg./day)*	*ACTH (25 mg./day)*
Number of obese mice	21	4	4	4	4	4
Average duration of survival and actual values	2.2 (0.5 to 5.5)	3.5 (3, 3, 3, 5)	14.3 (4, 12, 17, 24)	6.5 (4.5, 6.5, 7.8)	3.5 (3, 3, 4, 4)	0.8 (0.3, 0.7, 0.9, 1.3)

of decreased secretory activity. The lack of clear difference in the intensity of staining between the two outer zones in the obese animals was somewhat reminiscent of the pattern characteristic of immature mice. The zona reticularis revealed in the non-obese mice only a few lipid containing cells. There was no difference in the weights of the adrenals of the obese and non-obese mice.

The adrenals of both obese and non-obese animals exposed to cold for only a few hours, including the obese mice which died in this interval, showed no difference from those of unexposed mice. The adrenals of non-obese animals sacrificed after exposure to cold of seven days were enlarged and showed a histochemical pattern typical of the resistance phase of reaction to cold: slight increase in width of the zona fasciculata with cells more heavily laden with small droplets of sudanophilic, birefringent, Ashbel-

Seligman positive material, and, to a lesser extent, increase in positive lipid reactions in the zonae glomerulosa and reticularis.

In agreement with previous observations[3] no difference was noted between thyroid weight of these untreated obese and non-obese animals. In addition, the height of the thyroid follicle cells of obese and non-obese animals kept at room temperature were similar. Exposure to cold for a few hours had no detectable effect on cell height. Non-obese mice after seven-day exposure showed a significant increase in the height of thyroid follicle cells.

DISCUSSION

It is clear from the results presented that mice with the hereditary obese-hyperglycemic syndrome are unable to resist cold stress as successfully as normal animals. Increased sensitivity to cold has previously been observed in mice carrying the "yellow" obese gene.[24] This lack of resistance can, *a priori,* represent either a failure of the mechanisms regulating heat loss or a failure of mechanisms responsible for heat production.

Obese mice have an increased surface area probably not accompanied by a corresponding increase in number of hairs. This may lead to an increase in skin conductivity which may not be counterbalanced by the insulating effect of subcutaneous fat. It was difficult to decide on the basis even of careful observation whether there was any difference in pilo erection or shivering between obese and non-obese mice. No attempt was made to determine whether the obese animals, placed in the cold, were less able than non-obese animals to limit the flow of blood through their skin, tail, paws, and ears. If such an impairment did exist, it would obviously be an important factor.

A number of facts, however, argue in favor of ascribing the sensitivity to cold of obese mice to a failure of additional thermogenesis rather than to a failure of the mechanisms regulating heat loss. Young obese mice, with a near-normal surface area, already show decreased cold resistance. Non-obese mice which carry the "fuzzy" gene and have a very light fur coat display much greater resistance to cold than obese animals.* It appears likely to these authors that mice with the hereditary obesity diabetes syndrome are unable to raise their rate of mobilization and oxidation of reserve metabolites to permit successful resistance to a cold stress. While cold somewhat increases their spontaneous activity as compared to room tem-

* Extremely obese rats with hypothalamic lesions show hardly any decrease in resistance to cold. This syndrome is not characterized by a decrease in ability to burn acetate (report to be published). These findings, incidentally, would argue against Turner's interpretations of the etiology of "yellow obesity."[24]

perature rates,[5] it does not raise it sufficiently to make up for the deficiency in thermogenesis.

Glycogen reserves are low in the obese mice[3, 20] and blood glucose levels are particularly labile in the fasting state.[7] Fasting non-obese mice with depleted glycogen stores still resist cold considerably better than do non-obese mice. The utilization of stored protein does not seem to be implicated.[20] It is known that feeding of protein alone does not improve the resistance to cold of fasted animals.[6] It seems, therefore, highly probable that if there is failure in increasing utilization of reserves, it must be ascribed essentially to impaired mobilization or oxidation of fat. This, furthermore, agrees with the earlier demonstration of a partial block of acetate utilization in obese animals.[8]

While there is abundant proof that the adrenals and the thyroids are intermediaries in the physiological response to the cold stimulus,[1, 11, 12, 22] there is little likelihood that, in the present case, failure to resist cold is due to failure of the response of either the adrenals or the thyroid. Abnormalities of adrenal function have been eliminated as a primary factor in the etiology of this syndrome, since the obesity cannot be reproduced or ameliorated by adrenalectomy, cortical hormone, or ACTH administration.[20] The present studies, furthermore, show that at the time when obese mice die of exposure, the adrenals of non-obese mice do not yet show any morphological or histochemical evidence of increased secretory activity and that ACTH, normally toxic to the obese animals at room temperature,[20] seems also somewhat toxic in the cold. This argues against the possibility that failure of adrenal response alone can account for sensitivity to cold.

Similarly, as mentioned above, abnormalities of thyroid function are not implicated as a cause in the etiology of the syndrome.[3, 15, 19, 20] The extreme metabolic inertia of the adipose tissues has been shown to be responsible for the apparent hypometabolism in the obese mice.[7] While it is true that the weight of the obese animals can be reduced in part (though not to non-obese levels) by administration of large doses of thyroxine, the effect is limited in time. Furthermore, the higher doses of thyroxine required to bring about continued weight loss have a toxic and rapidly lethal effect.[20] In the situation considered here, very large doses of thyroxine, thyrotrophic hormone, and dinitrophenol give some degree of protection to cold, but this effect again is of short duration and appears to be palliative, and not directly curative. It seems likely, therefore, that the same block to fat utilization which is responsible for the obesity is responsible for the sensitivity to cold. While massive doses of thyroxine or allied substances may overcome to some extent or, more likely, circumvent this block, they do not seem to act on the reaction impaired by the primary block.

The results presented here appear to be of significance with respect to an old controversy in the clinical literature. The concept of "lipophilia" introduced by Von Bergmann and Hetenyi[9] implied that the adipose tissue of obese individuals is not mobilized normally. This theory has been denied by many workers. The gist of their arguments, as endorsed and summarized for example by Newburgh,[21] is that if lipophilia existed, obese individuals would not lose weight at the predicted rate when fasted. According to this reasoning, if fat is mobilized by severe fasting, then fat is normally mobilizable. On the other hand, the form of animal obesity studied here illustrates that normal weight loss during caloric restriction[20] may not be incompatible with impaired mobilization of fat under other conditions, such as exposure to cold. It becomes, therefore, questionable whether weight loss under conditions of caloric inadequacy is always an adequate test of the normalcy of fat utilization. For the obese mice to survive either during starvation or during exposure to cold, they have to overcome this block to fat utilization. In starvation, they are capable of doing this, at least for several days. In the cold it may be that they are completely incapable of doing so.*

SUMMARY

1. The hereditary obese-hyperglycemic syndrome of mice causes the animals to be extremely sensitive to cold and to die of exposure in a few hours. This is in contrast to the marked resistance of the non-obese animals, even when these are fasted, or fasted and exercised.

2. Pharmacological doses of thyroxine, thyrotropic hormone, or dinitrophenol prolong significantly, but not considerably, the life of hereditarily obese-hyperglycemic mice exposed to cold. Corticotropin shortens duration of survival under the same conditions.

3. Death occurs in obese animals before non-obese controls show any signs of cortical or thyroid hypertrophy.

4. It appears probable that sensitivity to cold is not due to an impairment of cortical or thyroid function but is due to a primary block in fat utilization. The concept of lipophilia is discussed.

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* Since this article was written, it has been demonstrated (J. Mayer, S. B. Andrus, and D. J. Silides, Endocrinology, in press; J. Mayer, Physiol. Rev., in press) that obese hyperglycemic mice are characterized by a primary hypersecretion of pancreatic hyperglycemic factor, causing a secondary hypersecretion of insulin.

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FORMATION OF TETANUS ANTITOXIN BY SPLEEN AND LYMPH NODE INTRAOCULAR TRANSPLANTS**

Evidence for antibody formation by various cells, tissues, and organs has come mainly from studies employing the following methods: (i) extracts of tissue cultures,[1-13] (ii) cannulization of afferent and efferent lymph,[14, 15] (iii) extracts of cells, tissues, and organs,[16-28] (iv) extirpation of the spleen,[29, 30] and (v) roentgen-radiation of animals with lead-protected mobilized spleens.[31] Although the reports arising from these are controversial, there now seems but little doubt that spleen and lymph nodes actively producing antibody in the intact animal will continue to do so for a few hours following removal and transfer to tissue culture. There is also fairly uniform agreement that the addition of antigen to a tissue culture does not increase, but may prevent, the formation of antibody.

The present study indicates that the anterior chamber of the eye of the irradiated mouse provides a good site for studying antibody formation by isolated tissue transplants. A distinct advantage is that the intraocular transplant is nourished in a more physiological manner than is possible with tissue culture methods. Tetanus antitoxin production by the transplants was studied because very small amounts of this antibody can be detected with good quantitative accuracy. The anamnestic response was employed to test the capacity of various intraocular transplants to produce this antitoxin. The advantage of employing this recall response lies in the greatly increased amount of antitoxin produced by a given quantity of antigen and its much more rapid production.

MATERIALS AND METHODS

Spleen and lymph node tissue from the donor was removed 30 to 60 days after the primary or secondary antigenic stimulus, as described in the various experiments. The tissue fragments, about 1 mm. in diameter, were transplanted into the anterior chambers of mice within one to three hours after irradiation. All recipient mice and irradi-

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ated controls in the experiments reported here received 600 Rep. whole-body cobalt-60 irradiation. This amount of irradiation normally causes no deaths in our four-week-old mice. The LD_{50} for mice of this age is 750 Rep. on our source. Irradiation of the recipient mice served the two-fold purpose of increasing the percentage "take" of the transplants and of practically abolishing antibody production by the recipient animals, thus increasing the probability of detecting the amount of antibody produced by the transplanted tissue. Six to 12 days after the transplants were established, the mice were injected intravenously with 0.2 ml. of a 1 : 4 dilution of fluid toxoid and 9 to 11 days later, they were bled. At this time, the mice with regressing transplants were discarded. The tetanus toxin* used in these experiments was ammonium sulphate precipitated and dried. A 2% solution of toxin was made in 50% buffered (pH 7.4) reagent glycerine and stored in ampoules at —18° C. If the precaution is taken always to use a dry syringe for removal, the toxin will remain stable for many months under these conditions and the original titration can be relied upon. Titration of the toxin was done in three stages: rough, semi-fine, and fine. Ten mice of our inbred strain were used for each dilution in the final titration. The antitoxin combining power was determined by comparing it to a standard obtained from the Institute of Health. The titration of the antitoxic serum from the various experiments was always done in at least two stages, a rough and a fine, and sometimes in three stages. This greatly decreases the number of mice required. We found the inbred strain of mice to be very uniform in their reactions to tetanus toxin and that two to four mice for each mixture gave very uniform results. Except for the very low unitage, the serum was varied and the toxin was held constant. For the very small amounts of antitoxin, it was more practical to hold the serum constant and vary the amount of toxin. All dilutions of toxin and serum were made in beef heart infusion broth. The titrations were read at the end of four days and we found it more accurate to use minimal paralysis instead of death as an end-point. However, the lethal end-point could be used without making a significant difference in the results.

EXPERIMENTAL

The first experiment was designed to determine whether tetanus antitoxin formation by lymph node and spleen intraocular transplants from immune donors into irradiated recipients could be detected. The donor mice had received one injection of 0.05 ml. alum-precipitated tetanus toxoid 30 days prior to the removal of the tissue for transplantation. These animals had from 0.125 to 0.250 International units of tetanus antitoxin at the time the tissues were taken for transplantation. The experiment was designed to test the formation of antitoxin by the transplant without further antigenic stimulation, as well as the effect of a secondary stimulus consisting of 0.2 ml. of a 1:4 dilution of fluid tetanus toxoid injected intravenously into the tail vein of the recipient irradiated mice 10 days following transplantation.

* We are indebted to Lederle Laboratories, Pearl River, New York, for a generous supply of this toxin.

From Table 1, it is clear that lymph node tissue which was given a secondary stimulus responded by producing tetanus antitoxin, whereas lymph node transplants not so stimulated did not produce detectable quantities. The spleen tissue possibly produced a very small amount after stimulation, and none without stimulation. Frontal sections through the anterior chamber of the eye, containing lymph node and spleen transplants, are shown in Figure 1 and Figure 2 respectively. These sections were taken seven weeks from the time of transplantation.

TABLE 1: TETANUS ANTITOXIN PRODUCTION BY LYMPH NODE AND SPLEEN TRANSPLANTS IN ANTERIOR CHAMBERS OF EYES OF IRRADIATED MICE

Spleen and lymph node donor tissues were taken from mice that had received only one injection of alum-precipitated tetanus toxoid subcutaneously 30 days prior to the transplantation

20 mice in each series received in a.c. of each eye	*Toxoid treatment 10 days after receiving transplants to a.c. of eyes*	*Fraction of Intern. unit ml. of pooled serum from group*	*No. of MLD of tetanus toxin neutralized by 1 ml. of pooled serum*
I—spleen	Not inj.	< .00004	< 1.5
II—spleen	Fluid tetanus toxoid i.v.	.00007	2.0
III—lymph node	Not inj.	< .00004	< 1.5
IV—lymph node	Fluid tetanus toxoid i.v.	.01	400.0

Because of the marked destructive action of the gamma irradiation on the lymph nodes and spleen of the recipient mice, the second experiment was designed to confirm the first one and also to test the possibility that spleen transplanted to one eye and lymph node transplanted to the other eye might have a stimulating or synergistic effect on each other. No increase in antitoxin formation resulted from a combination of lymph node and spleen transplants (Table 2). These mice had less antitoxin than those in Group II which had lymph node in the anterior chamber of each eye. It is also clear that the transplant must receive a secondary stimulus in order to produce a detectable amount of tetanus antitoxin. The amount of antitoxin produced by the mice in Group II is only 0.1 as much as in a similar group, Group IV in Table 1. The cause of this variation is not yet determined.

The very doubtful formation of tetanus antitoxin by the spleen transplants was somewhat surprising in view of the very definite production by

FIG. 1. Frontal section of lymph node transplant in anterior chamber of the eye seven weeks after transplantation. Stained with hematoxylin and eosin.

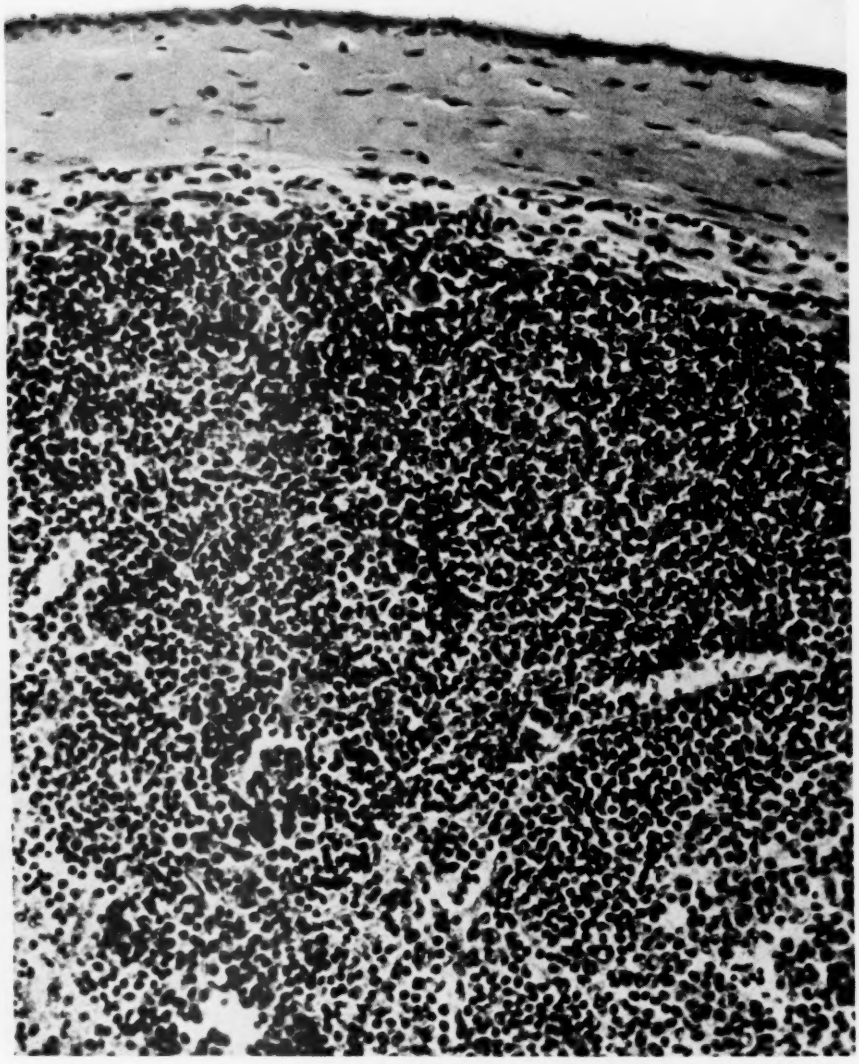

FIG. 2. Frontal section of spleen transplant in anterior chamber of the eye seven weeks after transplantation. Stained with hematoxylin and eosin.

the lymph node transplants. It should be emphasized here that in the first experiments the donor mice received only one subcutaneous immunizing injection of alum-precipitated tetanus toxoid. Under this condition, lymph node tissue definitely produced antitoxin, whereas antibody formation by splenic tissue was doubtful. The possibility that spleen from donors having had both a primary and secondary stimulus might be better for transplants of this tissue was tested in the third experiment. The donor mice were given

TABLE 2: TETANUS ANTITOXIN PRODUCTION FOLLOWING COMBINED LYMPH NODE AND SPLEEN TRANSPLANTS FROM IMMUNE MICE TO IRRADIATED MICE

Donor mice were immunized by a single subcutaneous injection of 0.05 ml. of alum-precipitated tetanus toxoid 55 days prior to transplantation

No. of mice receiving transplants	*Toxoid treatment 9 days after receiving transplants to a.c. of eyes*	*Fraction of Intern. unit/ml. of pooled serum from group*	*No. of MLD of tetanus toxin neutralized by 1 ml. of pooled serum*
I—20, no transplant Irradiated controls	Fluid tetanus toxoid i.v.	< .00004	< 1.5
II—25, lymph node*	" "	.001	40.0
III—25, spleen*	" "	< .00004	< 1.5
IV—25, lymph node in a.c. of one eye and spleen in a.c. of other eye	" "	.0003	12.0
V—25, lymph node*	Not inj.	< .00004	< 1.5

* In a.c. of each eye.

0.05 ml. alum-precipitated tetanus toxoid subcutaneously for the primary stimulus and 0.05 ml. of fluid toxoid intra-abdominally 22 days later for the secondary stimulus. The splenic tissue was removed for transplantation six days following the second injection. The donor mice having received two such injections had from 7.5 to 20 International units of tetanus antitoxin per ml. of serum 10 days after the second injection. Because of the antitoxin content of the donors' tissue, it was desirable to have a control on the amount of antitoxin carried over by the tissue transplants. For this purpose, twice-frozen and thawed splenic tissues from the immune donors were transplanted to the mice in Group III, Table 3. The recipient animals in Groups I and III were given 0.05 ml. fluid tetanus toxoid intravenously 12 days after receiving the transplants. Those in Group II were not injected

with toxoid, serving as controls for the amount of antitoxin formed by the live spleen transplants over a period of 12 days without further antigenic stimulus. Under the conditions of this experiment there is no doubt that the spleen transplants from twice-stimulated donors can form antitoxin in the irradiated recipients without any further antigenic stimulus if the donor tissue is taken during the active phase of antitoxin production. However, the intravenous injection of toxoid into the recipients more than doubled the amount of antitoxin produced. The antitoxin content of the serum from the

TABLE 3. PRODUCTION OF ANTITOXIN BY SPLEEN TRANSPLANTS FROM DONORS RECEIVING ALUM-PRECIPITATED TOXOID SUBCUTANEOUSLY FOR 1ST INJECTION AND FLUID TOXOID INTRA-ABDOMINALLY FOR THE 2D STIMULUS 22 DAYS LATER.
Twice-frozen and thawed spleen from similar donors was used as control. Donor tissue was taken 6 days after 2d injection

No. of mice receiving in a.c. of each eye	*Toxoid treatment 12 days after receiving transplants to a.c. of eyes*	*Fraction of Intern. unit/ml. of pooled serum from group*	*No. of MLD of tetanus toxin neutralized by 1 ml. of pooled serum*
I—25, fresh spleen transplants	Fluid tetanus toxoid i.v.	.00125	50.0
II—25, fresh spleen transplants	Not inj.	.00047	19.0
III—25, frozen and thawed spleen transplants	Fluid tetanus toxoid i.v.	.00004	1.5

mice in Group III indicates that only a very small amount of antitoxin is passively introduced by the frozen and thawed transplant.

Whole-body irradiation of the donor mice will greatly reduce the percentage and duration of "takes" of spleen transplants in irradiated recipient mice. Therefore, to control better this carry-over of antitoxin by donor tissue, an experiment was designed to use spleens from twice-stimulated, irradiated donors. The irradiated donors were given 600 Rep. cobalt-60γ whole-body irradiation one to three hours before the tissues were removed for transplantation. Spleens were taken from the immune donors 12 days after the secondary stimulus. The results of this experiment are represented in Table 4.

As can be seen, the serum from the mice in Group I and Group II—the recipients of the immune spleen from irradiated donors—had a just detectable amount of antitoxin. The injection of toxoid intravenously did not

increase the amount of antitoxin. The animals in Group I have no more antitoxin than those in Group II. In contrast to this, the mice in Group III with spleen transplants from non-irradiated immune donors and which had been given toxoid intravenously seven days after receiving the transplant had 0.001 International units of antitoxin per ml. of serum. The antitoxin content of the serum from the animals in Group IV is greater than in the control Groups I and II. Although this difference is not great, it exceeds the experimental error in titration, indicating that the spleen from non-

TABLE 4. COMPARISON OF ANTITOXIN-FORMING ABILITY OF SPLEEN TRANSPLANTS FROM IRRADIATED, ANTIGENICALLY TWICE-STIMULATED DONORS TO SPLEEN TRANSPLANTS FROM SIMILARLY IMMUNIZED, BUT NON-IRRADIATED DONORS

No. of mice receiving spleen in each eye	*Toxoid treatment 7 days after receiving transplants to a.c. of eyes*	*Fraction of Intern. unit/ml. of pooled serum from group*	*No. of MLD of tetanus toxin neutralized by 1 ml. of pooled serum*
I—19, from irradiated donors	Fluid tetanus toxoid i.v.	.00004	1.5
II—18, from irradiated donors	Not inj.	.00004	1.5
III—21, from non-irradiated donors	Fluid tetanus toxoid i.v.	.001	50.0
IV—21, from non-irradiated donors	Not inj.	.0001	4.5

irradiated donors, without further stimulation, and after having been transplanted to an irradiated host, continued to produce a small amount of antitoxin.

It may be well to point out that the splenic tissues were removed from the immune donors in this experiment 12 days after the secondary stimulus, whereas they were removed six days after such treatment in the experiment illustrated in Table 3. The more active antitoxin formation on the sixth day as compared to the twelfth day may explain the difference between the comparable Group II, Table 3, and Group IV, Table 4.

When donor mice immunized by a single subcutaneous injection of alum-precipitated tetanus toxoid were used in the first two experiments reported here, there was a clear-cut difference between the production by the lymph

node and spleen intraocular transplants. The results with lymph node were conclusive. The formation of antitoxin by splenic tissue under these conditions was doubtful. However, when this was used from donors that had received 2 immunizing injections, there was no doubt that these transplants could form antibody after being transplanted into the eyes of irradiated mice. Therefore, it was desirable to compare twice-stimulated lymph

TABLE 5: PRODUCTION OF TETANUS ANTITOXIN BY LYMPH NODE AND SPLEEN TRANSPLANTS FROM DONOR MICE THAT RECEIVED ALUM-PRECIPITATED TOXOID SUBCUTANEOUSLY AND 66 DAYS LATER RECEIVED FLUID TOXOID INTRA-ABDOMINALLY

Twice-frozen and thawed spleen was used as control on amount of antitoxin carried over by transplants from the immune donor. The tissues were transplanted 9 days following second injection of antigen

No. of mice receiving in a.c. of each eye	*Toxoid treatment 6 days after receiving transplants to a.c. of eyes*	*Fraction of Intern. unit/ml. of pooled serum from group*	*No. of MLD of tetanus toxin neutralized by 1 ml. of pooled serum*
I—24, lymph node	Fluid tetanus toxoid i.v.	.004	150.0
II—24, lymph node	Not inj.	.0009	37.5
III—24, spleen	Fluid tetanus toxoid i.v.	.02	830.0
IV—24, spleen	Not inj.	.0025	100.0
V—20, no transplant—Irradiated controls	Fluid tetanus toxoid i.v.	< .00004	< 1.5
VI—25, frozen and thawed spleen	"	.00004	1.5

and spleen tissue for their ability to produce antitoxin. The results of this experiment are given in Table 5. As can be seen, the amount of antitoxin produced by both the lymph node and spleen transplants is very much greater than in any of the previous experiments. This was true where the transplant was not stimulated as well as where an intravenous injection of fluid toxoid was given to the recipients 6 days after receiving the transplants. It is quite evident from an examination of this table that an antigenic stimulus greatly increases the formation of antitoxin by both transplants. One might conclude from this experiment that spleen was a much better antitoxin-forming organ than lymph node. We do not believe that such a conclusion should be made in view of the variable results obtained in the

different experiments. At present we cannot be sure what the reasons for this quantitative variation may be, but work in progress may at least partially answer it. Whether or not the lymph nodes used for transplanting are regional to the site of the first alum-precipitated toxoid injection may be one of the reasons. Unfortunately, no attention was given to this point in any of these experiments. We believe that the difference between the red pulp and the white pulp of the spleen may also account for the varying amounts of antitoxin produced by the splenic transplants. The recent work of Thorbecke and Keuning[6] would support both of these views.

One should not conclude from these experiments that the spleen is more difficult to sensitize than the lymph node, because the first sensitizing injections were always subcutaneous in these experiments. We are now trying intravenous, as well as intraperitoneal and subcutaneous, sensitization of the donors, in the latter case comparing the lymph nodes regional to the site with those remote from the site.

Two experiments have been done with intraocular transplants of thymus and Peyer's patch tissue from immune mice into irradiated recipients. One experiment was positive for both tissues and one was negative for both.

One experiment has been done with skin transplants. This tissue grows better in the anterior chamber than any other we have tried, but no detectable antitoxin was produced.

The M.L.D. of tetanus toxin and botulinus toxin is the same on a milligram for milligram basis. Because the molecular weight of tetanus toxin is 72,000 and that of botulinus toxin is given as 900,000, we believe that a much smaller amount of botulinus antitoxin can be detected than can tetanus antitoxin. We plan to compare the sensitivity of these 2 antigens for this purpose.

SUMMARY

1. Lymph node and spleen tissue from immunized donor mice will produce tetanus antitoxin when transplanted to the anterior chamber of the eye of gamma irradiated recipients.

2. More antitoxin is formed by the transplants from immunized donors when the recipients are injected intravenously with fluid tetanus toxoid.

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REGULATION OF FOOD INTAKE IN NORMAL RATS AND IN RATS WITH HYPOTHALAMIC HYPERPHAGIA†

Animals eat different amounts of different diets, which implies that they recognize some quality or qualities of the diet. Both Cowgill[15] and Adolph[1] were aware of this problem, but no one appears to have made a systematic attempt to define the dietary quality recognized by animals. Both of these workers were led to the opinion that their animals seemed to "eat for calories," i.e., for energy. However, the mechanisms by which animals achieve caloric balance have never been elucidated. Since Richter[34] has shown that a rat's eating is periodic, the total amount of food eaten must depend upon (i) the amount of food taken at each meal, and (ii) the frequency of meals. At the time of eating, some "intrinsic food factor"‡ must operate to produce a change in the animal which is the signal to the animal (conscious or unconscious) to stop eating. During this meal the intake of this factor up to a constant amount, or in an amount which will produce some constant change in the animal, will take precedence over other qualities of the diet. During the non-feeding interval some other change, or perhaps the same change in the opposite direction, must prompt the animal again actively to seek food. This concept does not imply that the factor is a *specific* component of the diet (in the chemical sense), although this possibility has not been ruled out. It seems perhaps more logical to expect that

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‡ What will be referred to by this term is not known to be a single factor, and the singular form is used only for convenience.

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some *general* quality of the diet, related to several or all of the components, is the important factor.

Other investigations have been concerned with the rôle of the stomach[12, 13] and of the central nervous system[24, 25, 33, 38, 42] in the regulation of food intake. It is now well established that bilaterally symmetrical lesions in the hypothalamus without anatomical or functional damage to the hypophysis can produce either overeating[5] or failure to eat[2] in experimental animals. The overeating leads to obesity as a result of positive energy balance produced by the large increase in food intake, to which decreased activity may contribute in a small way. The mechanism of this hyperphagia remains obscure, but it appears that it may represent the "consequence of a particular type of failure in the mechanisms which regulate energy exchange."[5] Since the appearance of a review of this subject,[5] the published reports of Brooks and his co-workers,[6–10] of Bruce and Kennedy,[11, 30] and of Miller, Bailey, and Stevenson[32] have further clarified the nature and certain of the metabolic consequences of this type of obesity.

The experiments reported here were designed to investigate the following: (i) the nature of what may be called the intrinsic food factor, which was studied by changing the diet in various ways and measuring the effect of these changes on three physiological variables, *viz.*, body weight, food intake, and water intake; and (ii) the rôle of the central nervous system in the physiology of the urge to eat, which was studied by comparing food intake after dietary changes in normal rats and in rats with hypothalamic lesions. The data obtained indicate that the intrinsic food factor cannot be any of the obvious dietary constituents, including caloric value. We suggest that this factor may be the specific dynamic action of the food. In rats with hypothalamic lesions the regulation of food intake in response to dietary changes was found to be abnormal, suggesting that there was a failure to respond to the intrinsic food factor.

EXPERIMENTAL STUDY

I. SHORT-TERM EXPERIMENTS IN NORMAL RATS

A number of variables complicate the interpretation of records of food intake in animals observed over a long period of time. For example, the metabolic state of an animal is influenced by its dietary history,[36] while food intake, in turn, is affected by the metabolic state.[31] In order to try to eliminate variables of this type, experiments of short-term duration were carried out, and the animals and their environment were kept as constant as possible. Some changes probably do occur within the animal during the

first 24 hours following change of diet, but for the purpose of these experiments such changes have been considered insignificant.

If food intake is regulated through the intake of some intrinsic food factor, there must be some definite quantitative relationship between the amount of food eaten, and either the quantity of the factor ingested, or the magnitude of the changes induced in the animal by the intrinsic factor. With a constant metabolic state, as in these short-term experiments, the animal's reaction to the food factor is presumably also constant. Consequently, the amount of food eaten should reflect in a quantitative way the intake of the intrinsic factor; and in spite of changes in composition of the

TABLE 1: DIETARY COMPOSITION: ADDITION OF FAT

Fat added	*Kcal./gm.*	*Per cent protein*		*Per cent carbohydrate*		*Per cent fat*	
% by weight	*(approx.)*	*Gm.*	*Kcal.*	*Gm.*	*Kcal.*	*Gm.*	*Kcal.*
0	3.0	20	24.0	56	67.0	3.5	9.0
29	4.7	14	11.0	40	32.0	31.0	57.0
57	6.4	9	5.0	24	15.0	59.0	80.0
86	8.2	3	1.5	8	4.0	86.0	94.5

diet, the intake of the factor should be expected to remain constant. In the following experiments, measurements were selected in an attempt to identify the intrinsic food factor by determining which component or quality of the diets was taken in a uniform quantity as the composition of the diet was altered.

METHOD

Sprague-Dawley and Yale strain rats, both male and female, were used for these experiments in groups of 12 to 60 animals. The average weight of the rats used varied from 200-450 grams, but the rats in any one group varied no more than ±20 grams from the group average. The groups were kept in a constant temperature room at 85° F. in large cages, 12 or fewer rats of the same sex to a cage, with *ad libitum* supplies of calf meal* and of water. Animals were removed from the large cages in groups of 8 to 24 rats of the same sex, strain, and size, and placed in small cages in this constant temperature room for a period of not more than 24 hours, usually 18 hours for convenience, where *ad libitum* food and water intakes were recorded following experimental manipulation of the diet by the addition of various substances. After each experiment the group was returned to the large cage and not used again for one week. Since a 7-24-hour period of fasting prior to the experimental procedures decreased the intragroup variability without influencing the major results, this procedure was employed in all but the initial experiments.

* Cooperative G.L.F. Mills, Inc., Buffalo, New York.

RESULTS

Varying amounts of fat (lard or olive oil) were added to the calf meal diets of rats, increasing the percentage of fat, the caloric density, and the "oiliness" of the diets. (The vitamin content, protein and carbohydrate content, and the content of other specific nutritional components were proportionately decreased. Table 1). The high energy value of fat produced large enough changes in the caloric density of these diets so that any

TABLE 2: FOOD INTAKE WHEN FAT WAS ADDED TO DIET

Lard added % by weight	*Number of rats*	*Initial weight (gm.)*	*Food intake Gm.*	*Kcal.*	*Gm. fat*
a. Sprague-Dawley Males (24-hour pre-fast).					
0	2		18.3 ± 1.3*	54.8 ± 3.7	0.64 ± 0.04
14	4		17.3 1.4	66.4 5.2	2.8 0.2
43	4		16.1 1.3	90.0 7.5	7.3 0.8
57	4		18.1 1.1	116.3 6.8	10.6 0.6
86	4		13.5 1.4	110.0 11.5	11.8 1.1
100	4	Approx. 300-325	11.6 1.6	104.6 14.0	11.6 1.6
0	4		17.5 ± 0.9	52.5 ± 2.8	0.6
43	4		15.0 1.6	83.5 8.9	6.8
57	3		16.0 1.3	103.0 8.1	9.5
71	4		15.8 1.1	115.0 7.6	11.4
86	4		15.1 1.2	123.0 9.6	13.0
100	4		9.5 1.1	85.5 10.5	9.5
b. Sprague-Dawley Males (no pre-fast).					
0	2	327	14.3	43	0.5
43	3	327	14.2	79	6.4
86	2	305	13.0	106	11.2

* Standard error of mean.

regulation of caloric intake should be easily discovered. Regulation of fat intake, as a specific nutritional component, could be studied simultaneously.

A total of seven experiments utilizing 108 animals was carried out (Fig. 1 and Table 2). In every experiment the *caloric intake* was above control level in each of the diets to which fat was added. Caloric intake was proportional to the amount of fat added up to 86%, beyond which there was a slight falling off of caloric intake. Maximum average caloric intake, in percentage, of control was 231% on 86% lard diets. The *gram intake* in each experiment was depressed, varying from a few per cent in 15% lard

diets to an average of 16% in 86% lard diets. (To maintain a constant energy intake, a 22% reduction in the 15% lard diet and a 63% reduction in the 86% lard diet would have been necessary.) The depression of volume intake was somewhat larger in each instance because of the relatively high physical density of fat, approximately twice that of calf meal (i.e., each gram of a high fat diet had a smaller volume than each gram of the control diet, in proportion to the amount of fat added). *Total fat intake* on the calf meal diet averaged 0.6 gram, and on the 86% lard diet averaged 12 grams. Changes in body weight were not recorded. The results obtained using olive oil were almost identical.

It was possible to ascertain that the varying "oiliness" of the diets did not influence the results by carrying out several experiments in which a non-nutritional oil was used to control this factor. Olive oil and mineral oil, or lard and vaseline, were added to produce a constant amount of added oil while varying the proportion of added fat. The inert oil, acting as a bulk substance, did not influence the effect of the added utilizable fat, and, as in the previous experiments, the caloric and fat intakes increased in proportion to the amount of olive oil or lard added.

Certain unsettled questions posed by these experiments were answered by later experiments (reported in detail below), *viz:*

a. The fairly low fat content (3.5%) of the stock diet (calf meal) raised the question of a specific fat hunger in these experiments. In later experiments of longer duration a calf meal diet was fortified with 5% added lard before a change to a 50% lard diet. The results on the first day after such a change were the same as those reported here.

b. The possibility that the added fat was excreted in the feces was considered. Feces of animals on high fat diets did not appear more greasy or larger in bulk than the feces of the controls. Data on fecal fat excretion obtained in long-term experiments indicated that the large caloric and fat intakes of the rats on high fat diets in these 18-hour experiments could not have been dependent on immediate or subsequent excretion of fat in the feces.

c. In the long-term experiments the diets were fortified with vitamins and minerals in several experiments. During the first day of these experiments the results were not different from those of the 18-hour experiments. It would seem unlikely, therefore, that vitamin or mineral deficiencies modified these results.

In experiments similar to those using fats, either water, a cellulose product (Fisher Ruffex), carbohydrate (dextrose, arrowroot starch, lactose), or protein (casein, purified pancreatic protein) was added to the calf meal diet of rats. When calf meal was diluted with two parts of water by weight, the caloric intake in the first 18 hours was 30% greater on the dilute diet than on the control diet. The 18-hour weight gain was also larger, and both of these values were statistically significant. With up to

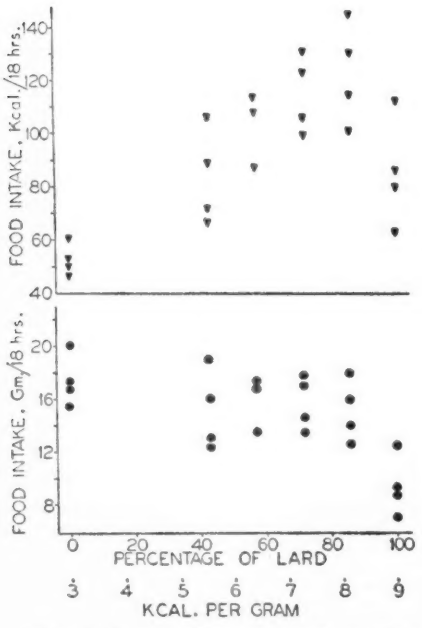

FIG. 1. In an 18-hour experiment, the caloric intake of rats receiving calf meal diets with added lard increased in proportion to the amount added until extreme levels were reached. Each point represents one rat.

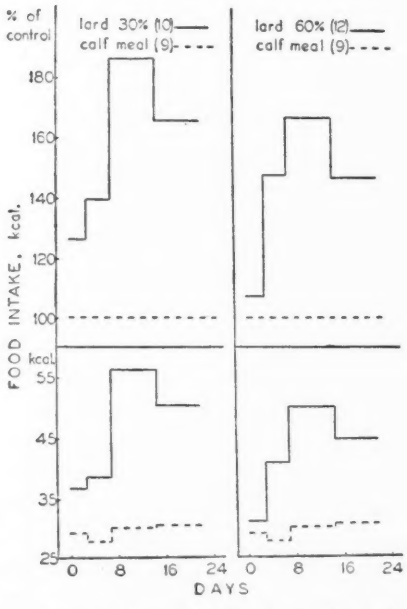

FIG. 3. When 30% lard or 60% lard was added to the diet of rapidly growing rats (9 to each group), they consumed a far greater number of calories than controls for the 22-day period.

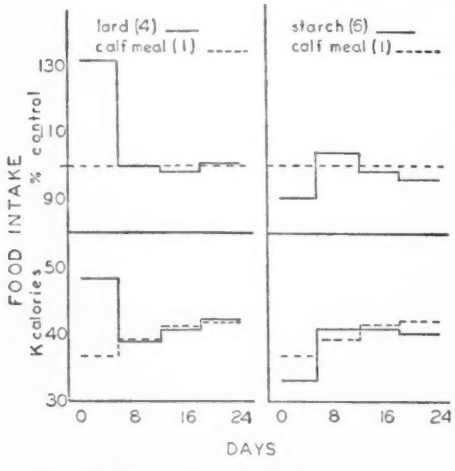

FIG. 2. When 43% lard or 43% arrowroot starch was added to the diet, the slowly growing rats (6 to each group) consumed the same number of calories as controls after a short time lag. In the first 5 days, the rats receiving 43% lard consumed 132% of the calories of controls; those receiving 43% starch, 91% of the calories of controls.

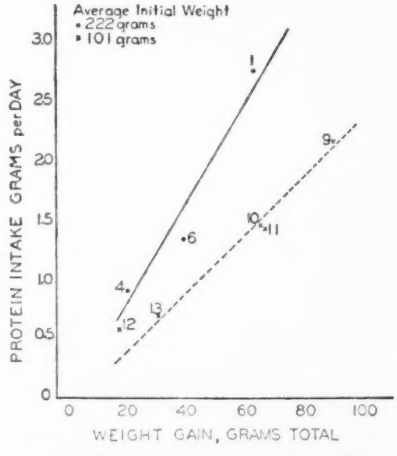

FIG. 4. The body weight gain of both rapidly growing and slowly growing rats was proportional to gram protein intake per day. The two separate curves obtained indicate that rapidly growing rats utilized protein for growth much more efficiently than slowly growing rats. Numbers refer to groups (Table 3).

15% added Ruffex (by weight) the gram intake was increased so that the intake of nutrients and calories as calf meal was maintained constant. At higher levels of added Ruffex, the increase of gram intake in this 18-hour experiment was not adequate to maintain constant caloric intake. The caloric intake of rats on diets containing up to 75% added arrowroot starch or dextrose was not significantly different from controls, and their gram intake was only slightly less than controls, a difference which was of borderline significance. Animals on 40% lactose diets consumed 78% of the calories of controls, a depression which was statistically significant. This difference between lactose and other carbohydrates has been observed previously[18] and has not been entirely explained. Rats on diets of 40% and 50% pancreatic protein or casein ate 88% and 61% respectively of the caloric intakes of controls, differences which were significant. The corresponding gram intakes were 78% and 53% of controls. In all of these experiments the specific nutritional component being added was taken in significantly increased amount.

II. LONG-TERM EXPERIMENTS IN NORMAL RATS

At the time the observations on food intake in the immediate 24-hour period following dietary change were being made, it seemed desirable to extend the observations in order to include data on food intake over a longer period of time after dietary change, and in order to study the adaptive phenomena which might occur. In the following experiments the food intake of rats adjusting to dietary changes over 22-24 days was studied.

METHOD

Two sets of experiments were run in successive months. In the first, slowly growing Sprague-Dawley male rats (average weight, 222 grams at the beginning of the experiment) were used. In the second, rapidly growing Sprague-Dawley male rats (average weight, 101 grams at the beginning) were the subjects. On arrival, each group was maintained, 12 rats to a cage, with *ad libitum* supplies of calf meal and water, for an acclimatization period of 10-14 days in a constant temperature room at 85° F. The rats were divided into groups on a basis of weight, 8 groups of 6 rats for the 222 gram rats, 5 groups of 9 rats for the 101 gram rats. Each experimental group was housed in the same room at 85° F. in its own large cage with *ad libitum* feedings of its experimental diet and continuously available water. On days 1, 7, 14, and 21 of the experiment, half of each group of 222 gram rats (i.e., 3 rats) had their food intake and water intake recorded for 24 hours (5 p.m. to 5 p.m.) by moving them to individual cages for this period. The other half of each group was treated similarly on days 4, 11, 18, and 24. The intakes for the 1st and 4th days were averaged, as were those of the 7th and 11th days, the 14th and 18th days, and the 21st and 24th days. All of these rats were weighed on days 0, 4, 10, 17, and 24.

The immature, rapidly growing rats were similarly handled. Food intake for half of each experimental group was measured on days 2, 4, 10, 17; for the other half, on

days 3, 5, 11, 18. Days 2 and 3 were combined, as were also days 4 and 5, 10 and 11, and 17 and 18, respectively. They were all weighed on days 0, 9, 17, and 22.

The basis for all diets was calf meal to which one of the following was added: Ruffex, water, lard, arrowroot starch, casein, and cane sugar. Supplementary vitamins and minerals were added to all diets. Composition and analysis of the diets to which fat or carbohydrate was added are given in Table 3.

TABLE 3: DIETARY COMPOSITION BY WEIGHT

	Diet number							
Ingredients	*1.*	*4.*	*6.*	*9.*	*10.*	*11.*	*12.*	*13.*
Calf meal	93	50	50	90	60	60	30	30
Osborne-Mendel salts	2	2	2	3	3	3	3	3
Cod liver oil	1	1	1	1	1	1	1	1
Yeast	4	4	4	6	6	6	6	6
Lard	..	43	..	..	30	..	60	..
Arrowroot starch	..	..	43	..	..	..	..	..
Cane sugar	..	..	..	..	..	30	..	60

DIETARY ANALYSIS			*Per cent protein*		*Per cent CH_2O*		*Per cent fat*	
No.	*Diet*	*Kcal./gm.*	*Gm.*	*Kcal.*	*Gm.*	*Kcal.*	*Gm.*	*Kcal.*
1.	Calf meal enriched	3	20.6	27	45	60	4.3	13
4.	Calf meal enriched plus 43% lard	5.7	12	8.5	24	17	45.8	74.5
6.	Calf meal enriched plus 43% arrowroot starch	3.5	12	14	67	78	2.8	7.5
9.	Calf meal enriched	2.9	21	29	45	60	3.3	10
10.	Calf meal enriched plus 30% cane sugar	3.2	15	18.5	49	73	3	8.5
11.	Calf meal enriched plus 30% lard	4.7	15	12.5	29	24.5	33	61
12.	Calf meal enriched plus 60% cane sugar	3.6	9	10	74.5	83.5	2.5	6.5
13.	Calf meal enriched plus 60% lard	6.6	9	5.5	14.5	9	62.5	84.5

RESULTS

Slowly growing male rats: Initial average weight, 222 grams. Forty-three per cent lard (Diet 6, Table 3) or 43% arrowroot starch (Diet 4, Table 3) was included in the calf meal diet of separate groups of rats for 24 days. Data for food intake and final body weight are given in Table 4 and Figure 2. The following results are evident from a comparison of the

three groups of adult rats: (a) The total gram intake of the groups varied in inverse proportion to the caloric density of the diets. The groups averaged the same caloric intake, although deviations from this average were noted during the first few days of the experiment. (b) Weight gain was independent of the caloric intake when the three groups were considered together. Caloric efficiency ratios (grams gained/day per kcal./day) were:

TABLE 4: EFFECT OF ADDED CARBOHYDRATE OR FAT ON FOOD INTAKE AND BODY WEIGHT

	Diets		
Results	*Control*	*43% CH_2O*	*43% fat*
Number of rats	6	6	6
Dietary protein			
% by weight	20.6	12.0	12.0
% by kcal.	27.0	14.0	8.5
Food intake			
Av./day for 24 days			
Gm./day	13.3	11.2	7.5
Kcal./day	39.9	38.9	42.7
Av. for days 1 & 4			
Gm./day	12.3	9.6	8.5
Kcal./day	36.8	33.5	48.5
Protein intake, gm./day, av. for 24-day period	2.74	1.34	0.90
Final body weight, gm.	285	261	242

Controls, 0.067; 43% carbohydrate, 0.043; 43% fat, 0.020. (c) Weight gain was proportional to the dietary protein concentration in calories, but not to the protein concentration in grams. (d) Weight gain was also proportional to the gram protein intake per day. Protein efficiency ratios (grams gained/day per gram protein/day) were: Controls, 0.97; 43% carbohydrate, 1.24; 43% fat, 0.92. These are roughly equivalent, except for a slight superiority of the 43% carbohydrate group which was, perhaps, related to utilization of some protein for gluconeogenesis in both of the other groups. The large supply of carbohydrate in the high carbohydrate diet would make it unnecessary for those animals to form glucose from non-carbohydrate sources, thus making a slightly increased amount of protein available for growth.

In parallel experiments, the calf meal diets of individual groups were diluted with 250% water, 43% casein, or 20% Fisher Ruffex. No significant difference in caloric intake or weight gain over the entire 24-day period was found under any of these circumstances, although both the gram and the bulk intake were proportionately increased on both the water and Ruffex diluted diets. The increase in caloric intake and weight gain reported above in the water diluted diets in the 18-hour experiments could not be

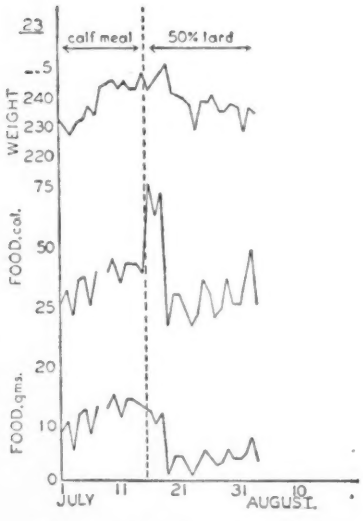

FIG. 5. "Caloric adjustment" occurred after a 3-day lag when 50% lard was added to the diet of a female rat. Rate of weight change was decreased.

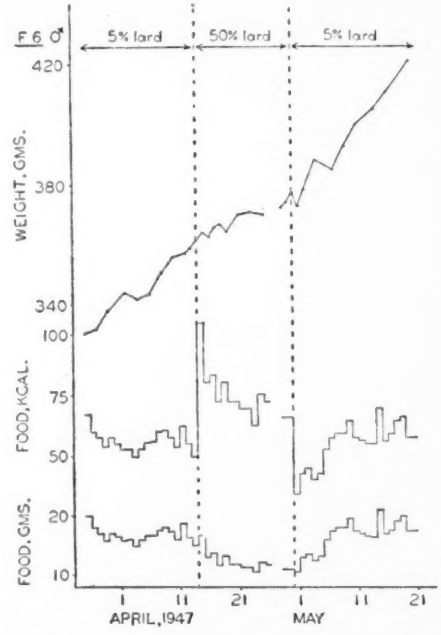

FIG. 6. "Caloric adjustment" occurred after a 3- to 5-day lag when 50% lard was added to the diet of a male rat. Rate of weight change was normal or slightly decreased.

found after three days. The rats given the casein diet were apparently able to utilize a high percentage of caloric energy supplied as protein without inefficiency. When a calf meal diet not enriched with vitamins and minerals was compared with the enriched control diet under these same circumstances, no differences in weight gain or food intake were observed, indicating that calf meal is probably an adequate diet with regard to these substances and the variables measured.

Rapidly growing male rats: Initial average weight, 101 grams. Thirty per cent or 60% lard (Diets 11, 13, Table 3) or 30% or 60% cane sugar

(Diets 10, 12, Table 3) was included in the calf meal diet of four separate groups for 22 days. Data for food intake and final body weight are given in Table 5 and Figure 3.

The following are evident from a comparison of these five groups of younger rats: (a) The gram intake of the groups varied considerably. The caloric intake of the rats on high carbohydrate diets was about equivalent

TABLE 5: EFFECT OF CARBOHYDRATE OR FAT ADDED TO DIET OF RAPIDLY GROWING RATS

	Diets				
Results	*Control*	*30% CH_2O*	*30% fat*	*60% CH_2O*	*60% fat*
Number of rats	14	9	9	9	9
Dietary protein					
% by weight	21	15	15	9	9
% by kcal.	29.0	18.5	12.5	10.0	5.5
Food intake					
Av./day for 22 days					
Gm./day	10.3	9.5	9.6	7.6	6.3
Kcal./day	29.3	30.8	45.3	27.3	41.5
Av. for days 2 & 3					
Gm./day	10.2	8.9	7.7	7.0	4.7
Kcal./day	29.0	28.8	36.5	25.0	31.0
Protein intake, gm./day, av. for 22-day period	2.15	1.43	1.44	0.69	0.57
Final body weight, gm.	192	167	166	132	118

to controls, but the rats on high fat diets consumed a far greater number of calories than controls. (b) The weight gain again was independent of the caloric intake when all five groups were considered. Caloric efficiency ratios (grams gained/day per kcal./day) were: Controls, 0.141; 30% carbohydrate, 0.097; 30% fat, 0.065; 60% carbohydrate, 0.052; 60% fat, 0.019. (c) Weight gain was proportional to the protein concentration in grams (omitting the 60% fat group for a reason discussed below), but not to the protein concentration in calories, in contrast to the results obtained with the more slowly growing animals when the reverse was true. (d) Weight gain, as in the case of the slowly growing rats, was also proportional to the gram protein intake per day. Protein efficiency ratios (grams gained/day per gram protein/day) were: Controls, 1.92; 30% carbohydrate, 2.10;

30% fat, 2.08; 60% carbohydrate, 2.05; 60% fat, 1.51. The group receiving the 60% fat diet differed markedly from the other groups. They received very little carbohydrate in their diet (Table 3) and probably utilized more protein for the formation of glucose.

In comparing the rapidly growing rats on high fat and high carbohydrate diets with the more slowly growing rats receiving similar diets, the following points are also evident: (a) In both instances the weight gain was proportional to the gram protein intake per day (Fig. 4) and to no other constant factor. The age and size of the animal influenced the efficiency of protein utilization (Fig. 4) as well as the availability of dietary carbohydrate previously mentioned. (b) The "caloric adjustment" of the slowly growing rats receiving a high fat diet was noteworthy (Fig. 2). This "adjustment" did not occur immediately, but occurred in time, within the first seven days after dietary change. The more rapidly growing rats on high fat diets took in many more calories than controls and did not make this adjustment of caloric intake (Fig. 3). The increased need of these animals for protein apparently affected the mechanisms of regulation of food intake, and is another example of the influence of the rat's metabolic state on the regulation of food intake.

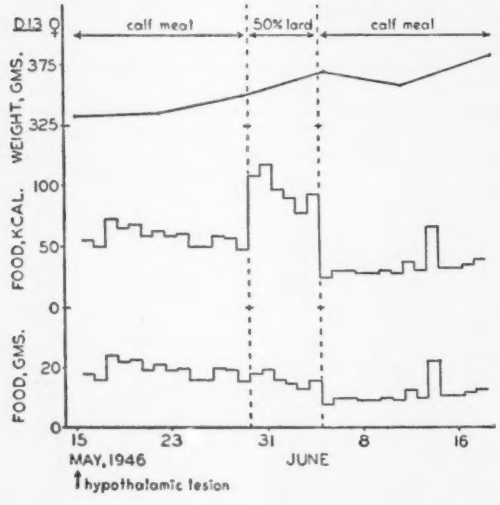

FIG. 7. When 50% lard was added to the diets of rats with hypothalamic lesions, their caloric intakes and rates of weight gain were increased. "Caloric adjustment" did not occur in the time studied. (D13 and D14 subsequently became very obese and D19 became slightly obese.)

A more precise definition of the adjustment to this type of dietary change was obtained by feeding 20 slowly growing rats on diets containing 5% lard during a control period, and then either 25% or 50% lard diets during an experimental period. The observed caloric adjustment occurred in from two to six days in 16 of the rats (Figs. 5 and 6). Four of the 20 animals failed to make the adjustment in the time studied (13-19 days). The rate of body weight gain was normal or slightly decreased in all the rats during

the feeding of the high fat diet. The maximum increase in the excretion of fat in the feces (determined by the method of Fowweather and Anderson,[22] which was measured on days 1, 2, 3, 4-7, 9, and 13 of feeding of the high fat diet, was 0.31 gram (2.8 kcal.) per rat per day for the female rats and 0.16 gram (1.3 kcal.) for the male rats.

III. RATS WITH HYPOTHALAMIC LESIONS

The experiments reported above define the types of regulation made by normal rats to certain experimental conditions. This section deals with experiments which attempt to define the type of regulation made by animals with hypothalamic hyperphagia under certain of these same conditions. Fat, cellulose, and water content were so studied.

METHOD

The female rats utilized for these experiments were kept in the constant temperature room at 85° F., on *ad libitum* calf meal and *ad libitum* water for at least two weeks prior to operation. Lesions inducing hypothalamic hyperphagia were placed with the Horsley-Clarke instrument by the method previously described by Hetherington and Ranson.[23] Rats used weighed from 200 to 325

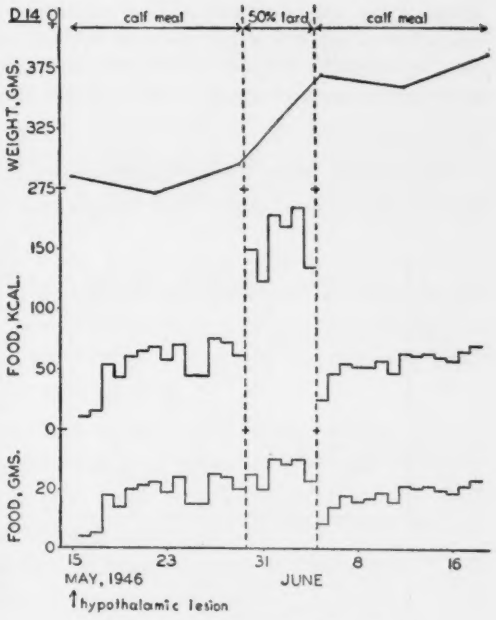

FIG. 8. See legend of Fig. 7.

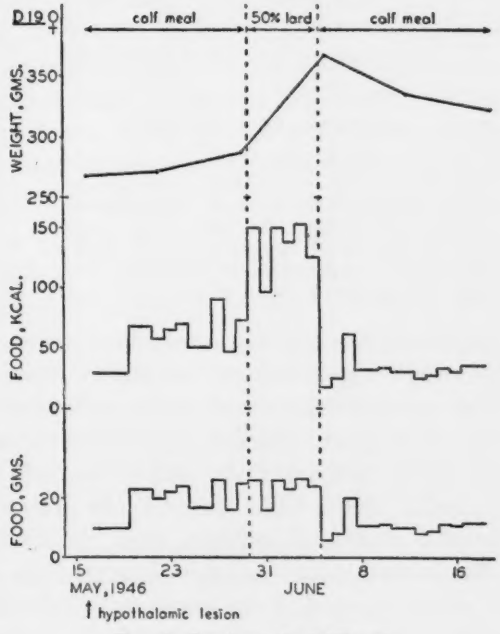

FIG. 9. See legend of Fig. 7.

grams at the time of operation. Postoperatively the animals were generally maintained on calf meal *ad libitum* and water *ad libitum*, and were utilized for experiments after varying intervals. Except as indicated, all of the rats which survived the operation were used in an experiment, whether or not they became obese.

RESULTS

Two weeks after hypothalamic operation, having been fed calf meal *ad libitum*, a group of eight Yale female rats was placed on a diet of 50% lard and 50% calf meal *ad libitum* for six days. Four of these rats subsequently became markedly obese, three slightly obese, and one lost weight. The caloric intakes of all the animals were increased when the diet was changed. The rats which later became the most obese took in 150-225% of the calories of their postoperative control period; this increased intake persisted for the entire six days with no evidence of a normal response during that time. The animals with less effective lesions showed tendencies toward the normal type of adjustment, but abnormally increased caloric intake always occurred. All of the animals, even the one with an ineffective lesion, showed an acceleration of the rate of weight gain, the degree being proportional to the degree of increase in caloric intake. Following refeeding of the calf meal diet, all of the animals showed a depression of food intake. Some of the rats recovered from this rapidly within three to six days, while others exhibited a very slow increase of caloric intake which never attained the level the animal had shown prior to receiving the high fat diet (Figs. 7-9). Additional observations[14] have revealed that a high caloric intake was maintained for as long as three weeks in several animals, while in the experiments of Lundbaek and Stevenson[31] it was maintained for as long as five weeks.

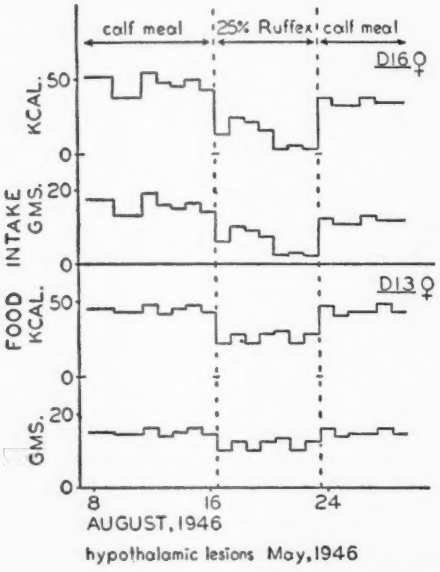

FIG. 10. When 25% Ruffex was added to the diets of rats with hypothalamic lesions, their caloric intakes were markedly decreased. These rats also lost weight rapidly during the period of feeding of the Ruffex diet. (D13 subsequently became very obese and D16 became slightly obese.)

After a period of one month on calf meal these same rats were presented with a diet of 75% calf meal and 25% Fisher Ruffex for six to seven days.

The volume of this diet was 1.7 times calf meal for equal weights. The gram and caloric intakes of all the rats were depressed during this period, and they all lost weight. On refeeding calf meal, with one exception, each of the rats immediately consumed the intake of calf meal it had had prior to the addition of Ruffex (Fig. 10). Since Adolph[1] has shown that there is a limit of bulk which the gastro-intestinal tract can handle, a partial explanation of these results may be that rats with large calf meal intakes reached the limits of their tolerance to bulk when Ruffex was added to their diets. However, since even the rats which were becoming obese but slowly and whose food intakes were in a more normal range did not increase their intake when Ruffex was added to the diet, some mechanistic fault, the result of the lesion, may also have been present.

When a diet of ground chow pellets diluted with an equal part of water (1:1) was further diluted with two parts of water (1:2) and then with three parts of water (1:3), the calculated dry food, caloric, and water intakes and body weight gain were unchanged for two obese rats, selected from a large group, and for their two normal controls.

DISCUSSION

These data describe the adjustments of food intake in normal rats after several kinds of dietary change. When fat, carbohydrate, or protein was added to the diet of rats, increased intake of each added component occurred. Several experimental situations in which normal rats did not maintain a constant caloric intake were found. For example, when slowly growing rats were fed diets of high caloric density, high in fat and sub-optimum in protein, they had in the initial period an abnormally high caloric intake; "caloric adjustment" to the level of caloric intake on the control diet occurred over a well-defined course of four to five days. In rapidly growing rats, abnormally high caloric intakes on high fat diets were observed throughout a 22-day experimental period. In the experiments of Cowgill,[15] the described "caloric adjustment" occurred in periods of eight days or longer in grown dogs receiving a high fat diet, more optimum in protein content than those of the present experiments. The present data, however, appear to exclude the caloric content *per se*, as well as the fat, carbohydrate, or protein content, as the intrinsic food factor which rats invariably recognize. We suggest that the specific dynamic action of the food may be an important intrinsic food factor, and have discussed this idea elsewhere, together with a new hypothesis of the mechanism of regulation of food intake (Strominger and Brobeck, Yale J. Biol., 1953, *25*, 383-390).

These experiments indicate, with those being performed at the same time by Adolph,[1] that dilution of the diet with water in moderate amounts has no influence on growth or on food intake of normal rats, although food intake is augmented for a very short period (greater than 24 hours and less than 3 days) following dilution. The contradictory results of Archdeacon and Allen[8] who performed the same experiment in dogs were obtained under conditions in which the daily feeding period was limited to 30 minutes. Under these conditions the calculated dry food intake on a wet diet (1:1) was greater than on a dry diet (1:0) when measured over a 15-day period.

The addition of a non-nutrient diluting substance, within limiting amounts, also appears to have no effect on growth, or on food intake exclusive of roughage. In these experiments a gradual adjustment to dilution of the diet with cellulose occurred during the first three to four days. These results are in conformity with those of Adolph[1] who showed that up to 25% roughage added to the diet produces no change in body weight, or food intake exclusive of roughage, but that 30% bulk may permanently interfere with the food intake of the rat. This conclusion is supported by other observers.[16, 29] Only under extreme and limiting conditions does bulk become an important factor in the regulation of food intake; consequently, under the conditions of our experiments, the rat must be recognizing some quality of the diet other than, or in addition to, bulk. Davis and Briggs[16] and Woolley and Sprince[44] substituted roughage for glucose and, for up to 20% roughage added to synthetic diets, found a slight increase in weight gain. Bearse, Miller, and McClary[4] substituted 12% roughage for ground corn in a mixture of natural foodstuffs and found a slight decrease in weight gain. These controversial reports may be ascribable to a change in the percentage composition of the diet in calories which in itself may positively or negatively influence food intake and weight change.

Why did the rats offered protein-deficient diets in these experiments, and those of many other workers, fail to gain weight at a normal rate? On *ad libitum* feeding, all the rats needed to do to obtain the additional protein required for growth was to consume more food. An important reason for the stunted growth was that the rats on these protein deficient diets stopped eating before they took in the amount of protein required for normal growth. In those experiments involving a suboptimum concentration of dietary protein, the body weight of the rats was governed by the total protein intake and influenced by the way in which the metabolic state of the animal affected the use of that protein; that is, it appeared to be influenced by factors which might determine whether the amino acids were to be utilized for protein synthesis, or deaminated and utilized for gluconeo-

genesis. Under these conditions, body weight was independent of caloric intake. Harte, Travers, and Sarich[26] and Hegsted and Haffenreffer[27] have noted that body weight gain and growth appear to be "accidents" associated with the food intake of the animal.

What did the rapidly growing animals on high fat diets do with the huge excess of calories which they took in over a long period of time? What did the slowly growing rats on high fat diets do with the excess they had in the first few days? (Actually, the slowly growing rats had an excess during the whole experimental period. They matched the caloric intake of controls throughout, but the controls grew more rapidly and soon became much heavier.) Some of the extra calories may actually have been stored despite the smaller weight gain. That is, the storage of fat at 9 kcal./gm. would produce the same weight gain as the storage of protein or carbohydrate at 4 kcal./gm. Thus, a difference in composition of tissues stored can account for apparent discrepancies in the number of calories producing equal weight gain.[37] While this may be a partial explanation,[41] increased fat deposition, or difference in appearance of tissues, has never been noted in carcasses of these animals. A second explanation is that these rats disposed of extra calories in the form of heat lost from the body, although incomplete data collected by us would indicate that large deviations, in basal body temperature at least, were not present.

Forbes, Swift, *et al.*,[19, 20, 21, 23] have found that on a constant protein intake the percentage of additional calories supplied as fat may in a small way influence weight gain; this is presumably related to increased efficiency of utilization (low SDA) of diets high in fat content. Ring[35] does not agree with this interpretation and believes that a high percentage of fat does not lower the SDA of diets. Lundbaek and Stevenson[31] and Deuel and his coworkers[17, 39, 40] also report the favorable influences of high fat diets on body weight. The diets of French, Black, and Swift[23] are similar in composition to Diets 10 and 11 of our experiments, and their results are in no way contradictory to those presented in Figure 4. When the animal is regulating its own food intake under conditions supplying suboptimum amounts of protein, the amount of protein it is able to ingest (and this is influenced by all the other constituents of the diet) appears to be the most important factor regulating weight gain. According to the above mentioned workers, under conditions providing equal amounts of protein, weight gain is enhanced if a high percentage of the non-protein calories is available in the form of fat.

Rats with hypothalamic lesions designed to produce hyperphagia increased their caloric intake when fed high fat, high caloric density diets

whether or not the lesions had been effective in producing hyperphagia. Their food intake was depressed when they were fed bulk diluted, low caloric density diets. The adjustments of food intake which occurred in normal rats in response to these dietary changes did not occur in rats with hypothalamic lesions. These data suggest that a physiological deficit in rats with these lesions may be a failure to recognize the change in the organism produced by the ingestion of food. These rats do, however, have the temperamental abnormalities which have been reported in cats with hypothalamic lesions,[48] and it is perhaps plausible that food preference or rejection might be responsible for some of the observed effects.

SUMMARY

1. Experiments designed to investigate the quality of food (intrinsic food factor) which is important in regulation of food intake have been performed. The dietary regulations made by normal rats following dilution of a stock diet with fat, carbohydrate, protein, water, and cellulose, both in the first day following dietary change and in long term experiments, are described. Increased intake of each added component occurred.

2. Following dilution of the diet with fat, caloric adjustment did not occur in the first day following dietary change in slowly growing rats, nor in either short or long term experiments in rapidly growing rats. These data appear to exclude caloric value as the intrinsic food factor which rats invariably recognize.

3. Rats with hypothalamic lesions deigned to produce hyperphagia did not adapt their food intake in the manner of normal rats when their diets were diluted with fat or with cellulose. These deficits in the regulation of food intake were present whether or not the lesions were effective in producing hyperphagia.

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A FRACTION OF COMMERCIAL YEAST WHICH PROTECTS AGAINST BACTERIAL INFECTION

A Preliminary Note

Since 1940 when we began a study of sensitization in laboratory animals treated with pertussis vaccine we have emphasized the rôle of the bacterial protein in this phenomenon. Considerable information has been accumulated which indicates that vaccination with *Hemophilus pertussis,* while increasing resistance against infection by the same organism will, at the same time, produce hypersensitivity to the *Hemophilus pertussis* antigen. Such sensitivity, however, is not specific for pertussis but crosses with several other species of gram negative bacteria.[1,2,3] Furthermore, mice immunized against pertussis are much more susceptible to infection by other gram negative bacteria than are their normal litter mates. Apparently, specific immunization can produce a non-specific lowering of resistance which predisposes the host to unrelated infections[4,5] and this may well be listed as another untoward effect of bacterial allergy.

Because bacterial protein has been shown to play a part in hypersensitivity and, from the above observation, is implicated in the etiology of infections, the search for an appropriate protein for desensitization was undertaken. The obvious selection was protein associated with the nucleic acid of the bacterial cell from the specific *Hemophilus* organism. Injection of this material reinforced the resistance of sensitized animals. However, since hypersensitivity is not species specific, the possibility of another source of comparable material was apparent and it was found that a commercial preparation of yeast sodium nucleate would not only reduce the hypersensitivity of vaccinated animals to the same pertussis antigen, but would make them refractory to gram negative bacteria. This logically lead to the possibility of protection for normal animals against similar infection.

Our numerous experiments prove that normal mice after injection with sodium nucleate are protected against infection by *Proteus, Pasteurella, Brucella* and *E. coli* organisms. Subsequent studies indicated that different brands of sodium nucleate were variable in potency; some were even inactive. Furthermore, the protective dosage of the active preparations ran as high as 100 mg./mouse. In search of the active principle we looked to the

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source material and undertook the fractionation of yeast. Although we did not identify the principle agent, we have succeeded in isolating a very potent fraction from a pressed cake of baker's yeast. Since the therapy of gram negative infections presents a persistent clinical problem, we have hastened to present these data in preliminary form. Details of the preparation and identification of the active material will be described later.

Briefly, it can be said that from 4 lbs. of yeast cake, 1.5 gm. of a dry material has been isolated which represents that particular yeast fraction

TABLE 1: SURVIVAL OF NORMAL AND SENSITIZED MICE* WITH 100 MG. SODIUM NUCLEATE
(Sensitized mice treated with *H. pertussis vaccine.*)

		Normal mice				*Sensitized mice*			
		Control— no treatment		*Protected— Na Nucleate*		*Control— no treatment*		*Protected— Na nucleate*	
Organism	*Dose*	*Survivors*	*%*	*Survivors*	*%*	*Survivors*	*%*	*Survivors*	*%*
Proteus vulgaris OX19	1.5 billion cells/mouse†	2/40	5%	45/57	78%	4/30	13%	49/65	75%
Pasteurella multocida	100 organisms/mouse	6/18	33%	26/41	93%	7/38	18%	9/20	45%
Brucella suis	2-4 billion organisms/ mouse	2/20	10%	16/20	80%				

* In every experiment mice were of the same age, sex and strain.
† This dose constitutes 1 LD for normal mice but 5 LD for sensitized mice.

containing protein and associated with biologic activity. Injection of as little as 0.3 mg. per mouse and 7.5 mg. per guinea pig protects these animals against a lethal dose of live Proteus. To contrast the enhanced protection provided by the yeast fraction with the protection afforded by active preparations of sodium nucleate we have grouped our data so that Table 1 shows the effect of 100 mg. of sodium nucleate against infection by one of three different organisms, and Table 2 shows the comparable protection provided by the yeast fraction in *Proteus* infections of both mice and guinea pigs.

From these data it is evident that commercial sodium nucleate in large doses and a new yeast preparation in small doses will protect animals against infection with gram negative bacteria. As far as we know yeast is

not currently used in the preparation of antibiotics although seven years ago Sartory and Meyer[6] reported the *in vitro* inhibition of *Proteus* by yeast macerate. In our experiments we could not confirm these observations. Moreover, we have found that sodium nucleate has no *in vitro* bacteriocidal or bacteriostatic activity whatever.

TABLE 2: PROTECTION BY THE NEW YEAST PRODUCT AGAINST *Proteus OX19* INFECTION IN MICE AND GUINEA PIGS

Test animal	*Dosage—Live* Proteus *organisms*	*Control— no treatment*		*Protected— new yeast product**	
		Survivors	*%*	*Survivors*	*%*
Guinea pig	25 billion	2/5	40%	5/5	100%
Guinea pig	50 billion	1/6	17%	7/8	88%
Mouse	2.5 billion	0/6	0%	15/18	83%

* 7.5 mg./guinea pig; 0.3 mg./mouse.

SUMMARY

Normal and sensitized mice are protected against the various gram negative bacteria tested by certain commercial preparations of sodium nucleate. In a search for a active component we have isolated from a pressed cake of baker's yeast a potent fraction which is over 300-fold more effective. Further work concerning the purification and identification of this yeast fraction is in progress.

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AN ABNORMAL INFERIOR SUPRARENAL ARTERY

A vascular anomaly found during the dissection of a human cadaver appears to be a unique anatomical variant. A search of the literature has not revealed a similar instance.

DESCRIPTION

The subject was a white male who died of a cerebral hemorrhage at sixty-six years of age.

The right internal spermatic artery, instead of having its origin from the aorta at the normal level, arose above that of the renal artery. It passed caudally between the right renal artery and the renal vein; from this point on, it had a normal descent to the testis. An anomalous vascular connection in the form of an artery 3 mm. in diameter, serving as the inferior suprarenal artery, arose from the internal spermatic artery immediately caudal to the renal vein. It passed anteriorly to the latter in its course to the suprarenal gland (Fig. 1). The right kidney was normal both in appearance and position.

DISCUSSION

In embryonic development, the vessels which will ultimately become the renal, suprarenal, and internal spermatic arteries are connected by many branches which constitute part of the periaortic plexus. It is possible that any of the connecting branches of the periaortic plexus may persist.[1] The present suprarenal artery arising from the internal spermatic was probably a connecting artery which persisted to become the inferior suprarenal. Such an origin of the inferior suprarenal artery is not unusual[2]; however, it is uncommon to find the internal spermatic and the inferior suprarenal arteries forming such an intimate loop around the renal vein. It is plausible to consider that in severe nephroptosis such an arterial loop could produce obstruction of the renal vein.

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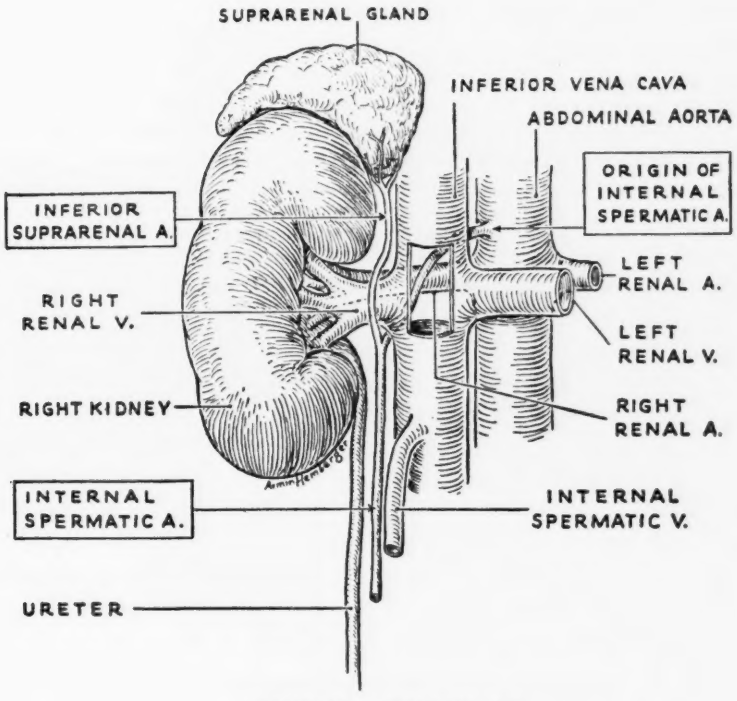

UNUSUAL ORIGIN OF
INTERNAL SPERMATIC ARTERY
AND
INFERIOR SUPRARENAL ARTERY

FIG. 1. An abnormal inferior suprarenal artery.

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ALBRECHT KOSSEL, A BIOGRAPHICAL SKETCH**

"The number of Bausteine which may take part in the formation of the proteins is about as large as the number of letters in the alphabet. When we consider that through the combination of letters an infinitely large number of thoughts can be expressed, we can understand how vast a number of properties of the organism may be recorded in the small space which is occupied by the protein molecule."[45]

Modern knowledge of protein chemistry rests firmly on a number of fundamental discoveries extending back to the time of Beccarius and Scheele. Although it has been two hundred and twenty years since Beccarius isolated gluten from wheat, factual knowledge of proteins has accumulated with great rapidity only during the last century. It may be noted that the impetus was not only the increasing general interest in science, but in part a recognition of the need for a new division of scientific learning, biochemistry. This daughter-science of chemistry and physiology has gained its recognized place through the efforts of many, among whom Hoppe-Seyler and Albrecht Kossel were pioneers.

Kossel was convinced of the necessity of relating chemical constitution to biological function. His particular field was the study of the nucleus of the cell, and he contributed much to advance early knowledge of the nucleic acids and two classes of proteins, the protamines and the histones. They formed his main scientific interests, and in order to characterize them he was led to a quantitative study of their composition and especially to the chemistry of the basic amino acids which are their principal constituents.

Kossel's† life and work were harmonious and his personality well fitted for scientific research. He was born on September 16, 1853 in the city of Rostock in Mecklenburg, the son of Clare and Albrecht Kossel. His father was a merchant, bank-director, and Prussian Consul in this Baltic seaport.[11, 15] The family was a large one of nine children: seven sisters and a brother, Hermann,‡ who was eleven years younger than Albrecht. The

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† His full name was Albrecht Karl Ludwig Martin Leonhard Kossel.

‡ Hermann Kossel received the degree of M.D. in 1888. He worked with Robert Koch from 1891 to 1904 and thus most of his researches were concerned with bacterial disease. He did outstanding work on diphtheria, malaria, and tuberculosis. In 1910, he went to Heidelberg as Professor of Hygiene. He died on April 30, 1925.[18, 87]

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Kossel family was sufficiently prosperous so that the young man's education was in no way handicapped.

Rostock, one of the most important commercial cities on the Baltic, had been thriving and influential from the time of the Hanseatic League and as a result of this leadership had secured a peculiar and liberal municipal constitution so that it existed more or less as a separate state until 1880. It may have been that the independent atmosphere of Rostock was an important factor in Kossel's frequently expressed dislike of the more typical Prussian spirit.

An old city, Rostock contains many picturesque Gothic buildings of which the most famous is the Rathaus lying in the center of the old town. Outstanding also are its five churches whose construction dates from the thirteenth and fourteenth centuries. One of these, St. Peter's, has a lofty tower which serves as a landmark to ships at sea. These towers were dear to Kossel, for in his later years he would often speak of them, and in the autumn of the year he longed to go North, for then the sunlight on the countryside shone as in Rostock.[16]

He attended the Gymnasium at Rostock and as a youth showed a keen interest in chemistry and botany.[15] His interest in botany led him to spend his first year as a medical student (in 1872 when he was nineteen years of age) at the University of Strasburg. These years at the university meant a separation from his family and an introduction to a new environment different from his northern German home; they also provided the opportunity to study with outstanding scientific men who had left other universities to come to head the faculty at the new university, which had just become German at the close of the Franco-Prussian War.

Here he followed his interest in botany by attending the lectures of Heinrich Anton de Bary, a physician and botanist, who was noted for investigations in cryptogamic botany. The formal courses offered by Bary included "Outlines of Botany," "The Propagation of Plants," and work in the botanical laboratory. His other teachers of this period included Waldeyer, who offered lectures in general anatomy and microscopy, Kundt, whose courses included experimental physics and electricity, and Bayer, the organic chemist, who taught "Organic Chemistry by Experimental Illustration" and offered practical work in the chemistry laboratory. Perhaps most important among his teachers was Hoppe-Seyler, who at Strasburg headed the first and for a long period the only department of biochemistry in Germany. Kossel undoubtedly attended both courses offered by Hoppe-Seyler—physiological and pathological chemistry, and a practical medical chemistry course.[8]

For some reason Kossel finished his studies at the University of Rostock, a much older institution than Strasburg. The University, like the city, must have been a singularly independent institution at the time of its founding in 1419 as it was conceived as a university without a faculty of theology, typically the center of medieval university life. Such a bold act soon had repercussions, for six years after the founding of the school a papal ban caused it to move to Griefswald. The faculty did not return to Rostock until eighteen years later.

The school had recently expanded its quarters when Kossel entered, and many of the buildings were only a few years old. Here Kossel completed his studies and in 1877 passed his state examinations in medicine. Such a change from one university to another was not an uncommon feature in the education of German students. The determination of the adequacy of the preparation of a student of medicine depended not on the number or sequence of courses taken but on the ability of the candidate to pass the state examinations. Nevertheless, most German students spent ample time on course work. Kossel, for example, studied five years before he felt prepared to take the examination. Instead of entering a clinic or preparing himself otherwise for clinical medicine, Kossel returned to Strasburg as an assistant in Hoppe-Seyler's laboratory; thus began his career as an investigator.

Probably his grasp of the importance of the quantitative aspect of biochemistry was derived from Hoppe-Seyler, who was an excellent inorganic chemist as well as biochemist and had made several contributions to quantitative inorganic chemistry.[70] Hoppe-Seyler was interested in the chemical components of tissues and was engaged in investigations on hemoglobin, which he had named, using techniques familiar to him as a chemist and also examining the absorption spectrum of hemoglobin and its derivatives. He realized that hemoglobin acted merely as an oxygen carrier and carried oxygen in the molecular form and concluded that the oxidation of nutrients occurred in the tissues rather than in the blood.

Kossel's first investigations dealt with diffusion and dialysis of salts and peptones,[27, 28] and appeared in volumes two and three of the *Zeitschrift für physiologische Chemie,* launched by Hoppe-Seyler—in June 1877—the year when Kossel came to work for him. Kossel became editor of the Zeitschrift after Hoppe-Seyler's death in 1895 and held the post until his own death in 1927, a period of thirty-two years.

In 1878, Kossel started the investigations of nuclear materials which were to remain his life-long interest. Hoppe-Seyler's concern with the constituents of the red blood cell had led him and one of his students to investi-

gate a phosphorus component which they isolated and named lecithin. In 1869, Hoppe-Seyler, as editor of *Hoppe-Seyler's Medizinisch-chemische Untersuchungen,* received a paper from Friedrich Miescher which announced the separation of a nuclear substance from the pus cell, to which Miescher gave the name "nuclein."[73] His work was not the result of chance but of a conscious effort to find a chemical explanation of the differences in the morphology and physiology peculiar to nuclear material.

The properties of the new substance seemed unusual as it had more strongly acidic properties than proteins were known to possess. It was soluble in dilute alkali and insoluble in dilute acids, water, and organic solvents, thus resembling mucin, although it was not mucin. It contained no sulfur, but considerable amounts of phosphorus were present. At the time, the only known phosphorus-containing organic components of tissues were lecithin and proteins.

These claims seemed incredible to Hoppe-Seyler, who did not publish the paper at once nor return it to its author, but repeated Miescher's work with the aid of two students, Plosz and Lübavin. When satisfied in 1871 of the correctness of Miescher's claims, Hoppe-Seyler published the papers of Miescher, the original paper on "nuclein" from pus cells and a second on "nuclein" of egg yolk.[74, 75] Other papers in this issue were by Hoppe-Seyler and his students on the isolation of "nuclein" from yeast and red cells.[24, 64, 78]

In Basel, Miescher turned to the isolation of the new material from the sperm of salmon and discovered that the nuclear material of the ripe sperm consisted of a salt whose basic constituent was a protein which he named protamine.

It was this heritage from Miescher, who was anxious to explain the nature of the nucleus, and from Hoppe-Seyler, who was interested in the phosphorus of the red cell, that influenced all of Kossel's future work. His first paper, on the nucleins of yeast, appeared in 1878, in volume three of *Zeitschrift für physiologische Chemie,* and a second paper followed a year later; these dealt with the preparation and analysis of nuclein and the isolation of the bases hypoxanthine and xanthine from its hydrolytic products.[29, 30] Since Piccard had isolated these purines from the nuclein of fish sperm,[77a] Kossel's papers were confirmatory.

Kossel made his first prediction concerning the nature of proteins in 1881. Salomon and Chittenden had been able to isolate the xanthine bases from blood fibrin and albumin, and the idea was growing that these compounds occur in all proteins.[8, 80] Kossel did not agree; he held that the xanthine bases were components of nucleins but not of the simple proteins. Unfortunately, he had no proof to support his statement.[31] During this year

Kossel, aged 28, became a privat-dozent, receiving fees from the students to whom he offered two courses: "Concerning sepsis and antisepsis" and "Clinical-chemical research methods."[10, 25]

In 1881, he published his first book, *Untersuchungen über die Nukleine und ihre Spaltungsprodukte*.[66] It was evident to Kossel that nutritional tissues such as the liver or spleen have a larger nucleic acid content than have locomotive organs such as muscle, and therefore this nuclear material was not a reserve substance but was formed during the regeneration of tissue.

In 1883, Kossel left Strasburg for a more responsible post at the University of Berlin under E. DuBois-Reymond, succeeding Baumann as Director of the Chemistry Division of the Physiological Institute. His teaching status was not changed, but he taught physiological chemistry, both by lectures and by work in the laboratory, in place of hygiene.[6]

He continued research on the nucleus and in 1884 published a paper on a protein isolated from the red blood corpuscles of birds which was in salt-like combination with nucleic acid. It appeared to be different from Miescher's protamine and Kossel named it histone.[32]

Kossel discovered adenine, which he first isolated from pancreas, in 1885. He also isolated it from yeast nuclein, described its chemical transformation into hypoxanthine, and the formation of its simple salts.[33, 34, 36] In the following year, he demonstrated that nuclein from egg yolk yielded no xanthine bases on hydrolysis and proposed the name "paranuclein" for the material.[35]

In 1886, Kossel married Luise Holtzman, the daughter of Adolf Holtzman, who had been Professor of German Literature and of Sanskrit at the University of Heidelberg and one of the outstanding philologists of his day.[2, 69] Luise Holtzman was an intelligent and charming woman and an excellent hostess. She had a wide knowledge of languages and was well read. The Kossels were very devoted to one another throughout life. Kossel enjoyed music and literature but they were allotted only a limited space in his life which was so filled with science and home that he had little energy left for pursuit of the arts.[15] A party at the Kossel household was an extremely pleasant affair. Kossel, generally shy and retiring, drew on his past experiences and was an excellent storyteller when settled in the pleasant and familiar atmosphere of his home.

The Kossels had three children of whom two lived to adult life, a daughter dying when very young. Their son Walther,* was born in 1888, and their daughter Gertrude in 1889.[12]

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* Walther Kossel is Professor of Physics at the University of Tubingen.[13] He is known for his theory of the physical nature of chemical valence and researches on x-ray and gamma-ray spectra.[13, 76]

In 1887, Kossel became Professor Extraordinarius in physiology. During this period he was busy with his new duties and with the publication of two books entitled, *Die Gewebe des menschlichen Körpers und ihre mikroskopische Untersuchung* (published jointly with Behrens and Schiefferdecker), and *Leitfaden für medizinisch-chemische Kurse*.[60] In the laboratory, he isolated theophylline from tea leaves and determined its structure and its relation to caffeine.[37, 38]

In 1888, Altmann announced his preparation of protein-free nucleic acid, and Kossel had the opportunity to study Altmann's product.[1] The results of his work were presented to the Physiological Society of Berlin on January 30, 1891.[30] Kossel announced the isolation of phosphoric acid, a considerable quantity of guanine and adenine, and a substance with the properties of a carbohydrate, from the products of hydrolysis of the nucleic acid. Two years later Kossel and his student Neumann isolated thymine and cytosine from "paranuclein," characterized thymine, and published a new method for the preparation of nucleic acids.[57, 58]

In January 1895, Kossel was called to Marburg to be Professor of Hygiene, but owing to the death of Kulz, he was appointed to the vacant seat as Professor of Physiology and Director of the Physiological Institute in April of that year.[21]

Marburg, in 1895, was a lovely town of 10,000 inhabitants. The University was the first to be founded as a Protestant institution in Germany; it was small, having only a few hundred students, but the faculty of medicine was distinguished and included Professors Korchelt in zoology, Hans Meyer in pharmacology, Schmidt in pharmaceutical chemistry, and Behring as Professor of Hygiene.[72]

Professor Kulz, Kossel's predecessor, noted for his work on sugar metabolism, had been a great friend of the Kultus Minister of Prussia, who built for him one of the finest laboratories of physiology in Germany. The laboratory was a stone building which contained a residence for the professor, with one or two rooms for the assistants, a library, an amphitheater, a laboratory with adjacent rooms for special techniques such as combustions, a professor's laboratory, and in the basement an animal room and a machine shop. The laboratory had desks for thirty to forty students and was used for both graduate and medical students. Kossel had seven graduate students at this time; T. H. Milroy from England, A. P. Mathews from the United States, Manilla Ide, Kutscher, who was later to be on Kossel's staff, Pierre Nolf from Belgium, Heine, and Noll.

Kossel offered courses in experimental physiology, general physiology, and the physiology and chemistry of urine secretion.[6] He had to teach the medical students elementary chemistry, as they were not given this instruc-

tion in the Gymnasium. Mathews reports that his lectures were clear and interesting.

At this time Kossel had the services of a personally trained assistant or diener, Petri, whom he had brought with him from Berlin. He, a native of northern Germany and an excellent technician, was responsible for arranging Kossel's demonstrations and carried out all his combustion analyses. Petri also looked after the students' supplies and even instructed the students in special techniques.

Kossel is reported not to have carried out an operation on an animal at this time.[72] But it is certain that he never lost his interest in carrying on his own chemical work. He is quoted by many of his colleagues as saying, "When I have discovered a new material, I will crystallize it myself."[10, 18] Like most chemists he had a special fondness for crystalline products and derivatives.

In August of 1895, Hoppe-Seyler died, and Kossel and E. Baumann, both students of Hoppe-Seyler, took over the editorship of *Zeitschrift für physiologische Chemie*. The first volume under their editorship (volume 21) contains an obituary written jointly by the two men, and the title of the journal was changed to *Hoppe-Seyler's Zeitschrift für physiologische Chemie*.[48] When Baumann died in 1897, Kossel carried on the editorship alone, which involved reading and criticising all of the papers submitted.

In 1896, Kossel isolated sturine and recognized its similarity to salmine.[40] This marks his entrance into the field of the proteins and nucleic acids of sperm, a work Kossel had taken up on Miescher's death. Kossel suggested that the term protamine should be used not only for the basic protein from salmon sperm, but as the name of a class of proteins. The isolation of the products of hydrolysis of protamines had led him to discover histidine as a mercury salt in 1895.[40, 55] Hedin independently isolated histidine as a silver salt in the same year. Kossel was so impressed with this work that he recommended Hedin highly for the vacant chair in physiological chemistry at the Jenner Institute in London.[10]

Probably the most important work produced during this period was the silver-baryta method for the determination of the basic amino acids, published in 1900 by Kutscher and Kossel.[56] The use of phosphotungstic acid, mercuric chloride, and silver nitrate had revealed to Kossel the possibility of a quantitative method to determine the basic amino acids. The method they developed was time-consuming and required a fair amount of material, but it was capable of producing accurate results if carefully handled. For many years it was the best method available for the analysis of basic amino acids.

Kossel's main interest now turned to the structure of proteins and in particular to that of the protamines. For years he sought an empirical formula for the protamines based on their amino acid content, thinking that they contained only basic amino acids without mono-amino acids, much as starch and glycogen are condensation products of glucose. This limited knowledge of the amino acid content of protamines led Mathews to ask Kossel (1895-1897) why he did not try to synthesize proteins from amino acids.[72] Kossel answered that he had considered the idea but had turned it over to Emil Fischer, who was better equipped to do that work and knew far more than he about organic synthesis. This does not indicate whether Kossel had considered seriously the mode of linkage between the amino acids which constitute a protein. If he had, he was not bold enough to state it publicly.

In a lecture to the German Chemical Society in 1901, Kossel discussed the position of protein chemistry and pointed out that the decomposition products represented many proteins, but that this did not mean that proteins were similar, for wide differences were found in the proportions of their various amino acids.[41] Kossel had shown that arginine is the main constituent of the protamines and that a large fraction of them consisted of basic amino acids, but he was never entirely satisfied with his methods and had considered the separation of amino acids through their esters. Dakin writes, "One of the bitter disappointments of his life was his failure to discover the ester method of separating amino acids. He supplied a student with adequate material but was assured that the higher amino acids did not form esters—a mistake he never forgave."[10]

He also continued work with students on the nucleic acids and in 1901, one of them, Ascoli, reported the discovery of uracil.[7] Evidently Kossel saw his graduate students often during this period, stopping by every afternoon to talk to them. Mathews found him a simple, friendly, modest, and kindly person. He describes Kossel as a fine-looking man about five feet ten inches tall, but who was often referred to as a tall man.

Mathews reports that he saw Kossel fussed but once.[72] DuBois-Reymond had died and the Berlin faculty was looking for a successor. The two people of choice were Kossel and Engelmann. The Kultus Minister, reported to be a stiff and arrogant Prussian, was coming to inspect Kossel and his laboratory. "We research men, the four or five of us, were stood at attention at our desks. Such a running around by Petri, Gerlach, the janitor, the assistants, and professor before he, the Minister, arrived you never did see!" Kossel did not receive the appointment, and Mathews remarks further, "Afterwards when Kossel went to Heidelberg, I asked him why he went there rather than stay at Marburg. He said he wanted to get out of

Prussia—he didn't like their domineering ways." Kossel told the following story himself: Kossel and his wife once took a trip to the Isle to Skye and were sitting on a bench enjoying the view. Kossel remarked, "Gott sei dank hier sind kein Berliner." Within a few minutes two Berliners sat down beside them.[10]

Whether or not this was the reason for his move, Kossel accepted in 1901 the chair of physiology and the directorship of the Physiological Institute at Heidelberg University that had become vacant on Kühne's death. This appointment was an honor because Heidelberg at the time had become the center of physiology in Germany. All of his students at Marburg travelled to Heidelberg with him,[22] so that when he reached there, he had a small group of graduate students which increased until in 1905, according to Mathews, there were thirty students from many lands. His lectures on physiology and physiological chemistry were attended on his arrival by one hundred students, and if the lectures became more popular as his fame increased, it is probable that the amphitheater (which seated only one hundred and fifty people) was always full.[77] He continued for a time to see his students rather frequently, but soon his visits were reduced to one or two weekly.[26] These students came from England, the United States, Russia, and Japan; in the early years at Heidelberg they were entertained informally at the Kossel home on the second floor of the Institute, but in later years these occasions became more formal.[77]

Kossel's lectures were always well prepared and up to date with the literature[9] and he used lantern slides and charts extensively.[77] He was not by nature a gifted speaker, and it speaks well for his preparative work that his students thought the lectures clear and interesting. To quote S. Edlbacher,[35] who worked with Kossel for fourteen years, "He spoke publicly with evident strain. It appeared to be a touch of modesty, against which he struggled until the last years of his life. He, therefore, spoke unwillingly at congresses and discussions." This shyness and reticence with strangers made him sometimes appear formal and stiff. He had a pleasant sense of humor and when telling stories his eyes twinkled with merriment and appreciation of the climax.[79] He spoke particularly well about the years he assisted Hoppe-Seyler, a period of his career which he remembered with great affection.[16]

His reserve seemed to detach him from everyday worries and troubles, and he solved his problems with an outward calm. Edlbacher, one of his students, comments, "In the fourteen years of almost daily contact, I never saw him (Kossel) annoyed. His equanimity invited the greatest respect."[18]

During this period Kossel sought to elucidate the structure of proteins by the following steps: (a) determination of the composition of the frag-

ments of hydrolysis, (b) the quantitative determination of their proportions, and (c) the mode of combination and arrangement of these fragments.[46]

He was not deluded into thinking that this would be simple; he stated the complexity of the task to Edlbacher thus: "I think of the protein molecule as one which can always answer to a chemical attack with some one of its characteristic groups. Much as grapes hang on a vine, so the protein molecule possesses a large number of characteristic groups; the guanidino group, the imidazole group, the indole nucleus, and so forth; perhaps necessarily in a given special combination, but they lie there in a manner which is accessible to attack."

Kossel considered the complex chemical constituents of the cell to be formed from simpler compounds which he termed Bausteine. These primary units were present in all living cells and from them were built by the processes of metabolism the secondary building stones which vary from organism to organism. These ideas were placed on a sounder basis when at Kossel's motivation Tamura carried out an analysis of bacterial cells and found that these small beings contained all the normal protoplasmic Bausteine.[53–56]

His view of the constituents of the cell was by no means that of it being a static system. He and his students showed that as the sperm matures, the proteins increase their content of basic amino acids. He spoke of this process as a step-by-step degradation of a more complex protein, containing a whole array of amino acids, to a simpler structure such as the protamines which contained relatively few amino acids. With F. Weiss he demonstrated that the muscle lost by the salmon contained sufficient arginine to account for the large accumulation in the maturing sperm and suggested that the other amino acids from the degraded muscle served as energy sources.[58] With Schenck, he investigated the testicle at varying stages of maturation and showed the increase in the content of basic proteins.[59]

Kossel tried to find some unit which might repeat itself in the protamines, by the silver-baryta method and Fisher's ester-distillation method. He and Dakin found the following proportions of amino acids in salmine:

Arginine nitrogen		89.2 per cent
Serine	"	3.25 "
Valine	"	1.65 "
Proline	"	4.3 "
Loss	"	1.6 "

The molecular proportions would then correspond to ten molecules of arginine, two of serine, two of proline, and one of valine.[51]

Kossel suggested the following method for subdividing the protamines. If arginine is denoted by *a*, histidine by an *h*, lysine by an *l*, and monoamino acids by an *m*, and the relative amounts of these individuals by a number, a monoprotamine would contain only arginine as its basic component and could be represented by *am*, a_2m, or a_3m depending on the relative proportions of the arginine to the monoamino acid, or if it contained more monoamino acids than basic amino acids, as cyclopterine, by am_2. There would be two diprotamines: (a) those containing arginine and histidine and (b) those containing arginine and lysine. A triprotamine would contain arginine, histidine, and lysine; for example, sturine, which is represented as $(ahl)_2m$.[47]

Arginine was always of particular interest to Kossel. Hence it is not surprising that he and Dakin should discover arginase in liver;[50] the discovery was, however, somewhat accidental. Dakin had worked previously with Hedin on kidney autolysates but even with his help had been unable to find arginine. Kossel supplied Dakin with arginine carbonate and suggested that liver might be a better source than kidney. They were amazed to find that the blanks lost arginine rapidly, in five minutes or less, at this date an incredible speed. They showed that the arginine was converted enzymatically to ornithine and urea. Kossel also found a second intermediary product of arginine, agmatin, produced from arginine by decarboxylation.[42, 43]

One other enzyme, nucleinase, was studied by Kossel and his student de la Blanchardière in 1913.[14] They observed that certain tissue extracts caused a decrease in the viscosity of the nucleic acid solution before any chemical decomposition occurred.

Researches with Kennaway, Hiryama, and F. Weiss showed that arginine is bound in proteins by the α amino and carboxyl groups and that the guanidino group is free.[23, 54, 63] The same result was shown in studies on the imidazole group of histidine and the ϵ amino group of lysine.[49, 52]

Then Kossel turned to the isolation of peptides from his protamines. He studied the optical rotations of proteins and found evidence that treatment with alkali caused racemization while the amino acid was bound in the protein.[60–62]

He was interested as early as 1901 in crystalline derivatives of the basic amino acids, and asked Dakin to select compounds which he thought might yield such derivatives.[10] It was a fitting close to his life's work that he and E. Gross should have discovered (from a large number of aromatic compounds supplied by the Badischen Anilinfabrik) that dinitro-naphtholsulfonic acid, which Kossel named flavianic acid, formed an insoluble salt with arginine.[53]

To his credit, many of Kossel's students continued to work on problems in which he was interested. Levene, a student at Marburg, was a leader in the field of the nucleic acids and Dakin in amino acids and proteins. Steudel and W. Jones added to our knowledge of the nucleic acids. Folin not only advanced an important theory of protein metabolism but introduced methods of assay for tyrosine and tryptophan which enabled one to work with extremely small quantities of material. Many of Kossel's students such as Mathews, Hart, Henderson, and Cameron became outstanding investigators and as well able teachers. Kossel did not appear to be motivated in his work by the desire for personal acclaim but rather by curiosity and the satisfaction gained from the discovery of scientific truths. He was invariably modest about his own contributions and could not understand the base motives occasionally revealed by other scientific workers.[15] It has been said of him that he did not set his name on a publication unless he had contributed to the work[16]; a number of his students published papers from his laboratory on which his name does not appear. In the latter part of his life he was considered without doubt the leading German biochemist both by his own countrymen and by those abroad. The list of his honors extends from 1904, when he received an honorary Doctorate of Science from the University of Cambridge, up to the time of his death.*

From 1907 to his death he held the title of Geheimrat which placed him on the board of ruling members of the University of Heidelberg and assured him his position there for the remainder of his life. In the year 1908 to 1909, Kossel directed the business affairs of the University as Prorector.[15] On August 12, 1907 he opened the Seventh International Physiological Congress in Heidelberg, the first meeting of this group held on German soil, and, as host, gave the opening address welcoming the members.[19] This was a period of festivity for visitors but was devoted also to the more serious interchange of ideas; entertainments were most pleasant and included a trip in gaily decorated boats down the Rhine from Mannheim in the evening. On approaching Heidelberg, the castle was emblazed in a flood of light from fireworks. At the close of the meeting it was unanimously voted that each member of the congress would send a photograph to Kossel as a souvenir.

In 1910, he received the highest honor given to scientific men, the Nobel Prize in Medicine, for his work on the nucleus of the cell. The prizes were

* Kossel received honorary degrees from the University of Greifswald (1906), from the University of Dublin (1908), from the University of Geneva (1909), and from the University of St. Andrews in Scotland (1912). He was made a member of scientific societies all over the world, including those in Stockholm, Upsala, Turin, Washington, Copenhagen, Gottingen, Leningrad, and Heidelberg.[9, 12, 21, 82]

presented by the King of Sweden on December 10, 1910, and amounted to £8000 each.[3] Kossel delivered on December 13 his statutory address, *Uber die chemische Beschaffenkeit des Zellkerns.*[4] The presentation of this award was followed by festivities at Heidelberg. The Student's Corps of the University honored him with a *Fackelzug,* or torch procession, and Kossel gave a party which was attended by his students, a number of the professors, and other academic guests.[26]

Probably Kossel was the honored guest at many German chemical and biochemical meetings, and he must have had to rely more on the help of his associates for the conduct of the routine academic duties. In particular, Petri was given more responsibility. He was devoted to Kossel, but like many persons of his status who have been given supervisory responsibilities, he began to consider that it was he who was actually in charge. Often Petri glorified his own wishes with the false statement that the Geheimrat wished it so, and his arrogance at times made life miserable for the students.[20, 72]

In the autumn of 1911, Kossel was invited to give the Herter Lecture at Johns Hopkins. He chose as his title, "The Proteins."[16] This was the only time Kossel came to the United States. It was a welcome opportunity for him to visit his many American friends and relatives, as well as those in this country with similar scientific interests. In preparation for the trip, he employed a woman student, Ruth Skelton, to tutor his daughter and himself in English conversation.[61] He knew the language slightly, for he could converse in English with his students when necessary. As part of the course of instruction, Miss Skelton read the *Pickwick Papers* to him. Kossel enjoyed them immensely and laughed heartily at the low-comedy characters like Sam Weller.[26] That Kossel enjoyed Dickens is not surprising, for his favorite German writer, and one whom he quoted fluently,[15] was Fritz Reuter, a man not unlike Dickens in many respects. Reuter wrote in Platt-deutsch about the people of Mecklenberg; he was an excellent humorist and a homely philosopher to the core. His characterizations rather than his plots made his stories, poems, and dramas real and full of a warmth particularly dear to his countrymen.

Kossel's wife, whose English was much better than his, found a book in this language on proteins and amino acids as source material for him.[26] His conversational English was evidently satisfactory; he stayed with the Dakins before the Herter lecture and several times used them as guinea pigs to test his skill. A guest, L. F. Barker, said that no German he had heard spoke English so well.[10] Dakin translated both his Herter and Harvey lectures into English for him. The Harvey Lecture was delivered in German on October 14, 1911, and was entitled "The Chemical Composition of the Cell."[45]

Kossel was an excellent guest and a very accommodating person. When Dakin apologized for the ugliness of the Third Avenue Elevated in New York, Kossel replied, ". . . But it gives shade!"[10]

He visited and gave lectures at several other universities, including the University of Chicago. A. P. Mathews, his friend and former student, was the head of the Department of Biochemistry at Chicago at the time, and the Kossels spent a pleasant week there.[72]

Kossel's wife had a number of relatives in this country who were very prominent citizens. These cousins were all members of the Hilgard family. Eugene Waldemar Hilgard was one of the leading geologists of this country in the latter part of the nineteenth century. In 1911, he was a professor emeritus of agricultural chemistry at the University of California in Berkeley, and the director of the agricultural experiment station in that state.[67, 69] Mrs. Kossel took back to Germany a rocking chair which he gave her, and it became the seat of honor in their home.[77]

Another cousin, Henry Villard (whose name had been changed to Villard from Hilgard), was a journalist, newspaper owner, and a financier of railroads and the Edison General Electric Company. His wife, Fanny Garrison Villard, was the only daughter of William Lloyd Garrison.[68]

The following years were sad ones for Kossel. His greatest loss occurred in 1913, when his wife died of acute pancreatitis. The impending war added to his sorrows. He was apparently not greatly interested in politics and seldom talked about them, although he is thought to have supported the more liberal Social Democrats. He never felt the necessity of maintaining "Germany's place in the sun."[10] His wife, who was undoubtedly more nationalistic, could embarrass him by mentioning this subject to guests at the dinner table. Kossel did not sign the Pronunciamento of German professors at the start of the war.[71] Perhaps the best description of his feelings during this period is that of his student, Edlbacher.[15]

> Kossel's life ran quietly. Difficult crises did not appear in his inner or outer nature . . . the World War with its consequences weighed on his soul, but his character was much too balanced not to overcome such blows of fate. The author knew him personally in 1913, when a great melancholy emanated from Kossel, as he still felt the loss of his wife . . . but a deep humor had helped him to surmount these years.
>
> The war had weighed heavily on his mind. Being aware of Germany's intellectual isolation, he realized, more than others, the plight of the fatherland. Certainly he, who had lived in harmony with the whole scientific world, must have felt strongly the breach of such a union. . . . Kossel felt very strongly from the start that the aggravating submarine warfare would bring America into the war.
>
> His strong love of truth suffered under all the confused mass of slander and lies which filled the world at that time. He was summoned, for example, each year to high

places to excuse official acts, by pronouncing that the allotted food provisions were sufficient. He refused this demand and others; still he was asked each time for a testimony, but would never declare untruths as truths.

Kossel still felt humble about Germany's rôle in the war in 1923, when he was honored as Germany's leading representative to the Eleventh Physiological Congress in Edinburgh.[20] Here he was given an honorary degree by the University, and when he appeared on the program of the Congress, he was greeted by applause lasting several minutes.[65] He was the guest of the president of the Congress, Sir Edward Sharpey-Schafer, who at this time was the sole surviving member of the original group that had founded the British Physiological Society and had acted to form the International Congresses. It is not surprising that Kossel was greatly pleased when Richet, a leading French physiologist, greeted him. Kossel realized that he might well have been passed by and said to Mathews, "You saw that he greeted me first!" It is reported that Kossel on his return to Germany again expressed his gratitude at the acclaim and recognition he had been granted by the members of the Congress.[65]

That year, 1923, marked Kossel's seventieth birthday, and in commemoration of the event, volume 130 (1923) of the Zeitschrift contained his picture near the titlepage, and the volume was dedicated to him.

In 1924, Kossel became professor emeritus, but he did not retire from active work. He continued to deliver his lectures in physiology and physiological chemistry.[6,17] He was fortunate in being able to carry on active research work at the Institute of Protein Research at Heidelberg which he had helped to found as the gift of the manufacturer, Fritz Behringer. Kossel had been the Director of the Institute, which was part of the laboratory of the Medical Clinic. On his retirement, he was asked by the Director of the Clinic, L. v. Krehl, to continue his research there.[15]

Probably the last scientific gathering Kossel attended was the Lister Centenary Celebration held in England in April of 1927.[5] He and the other delegates heard Sir E. Rutherford, President of the Royal Society, present the opening address to the King. In His Majesty's reply the hope was expressed that this gathering of scientific men would "strengthen the cooperation of all nations in the accumulation of scientific knowledge for the benefit of the human race." For Kossel this somewhat platitudinous message must have had a special meaning.

As the last years of Kossel's research were crowned with the discovery of flavianic acid, so his last months were spent in the labor of writing a monograph on the *Protamines and Histones,* which was published shortly after his death on July 5, 1927. Late in June he had an attack of angina pectoris which recurred on July 4, and he died quietly on the following day.

In the preface to his monograph he summarized the contributions made to that date by the researches on the chemistry of the nucleus. He felt that our knowledge "has so far been derived only from the descriptive side" and that the substances found in the nucleus were so far purely of chemical interest. However, the wide variety of chemical structures in the various species and genera of animals suggested that a more important morphological distinction might be found to result from these chemical differences. Kossel reiterated the hope that the protamines, as analogues of typical proteins, might be particularly favorable substances for the study of certain properties and structural relations of the proteins.

Thus Kossel in his seventy-fourth year was to the last still looking forward to new experiments which might solve some of the mysteries of the important rôle of the nucleus in the development of the cell and of the organism.

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BCG VACCINATION. Studies by the WHO Tuberculosis Research Office, Copenhagen. Report prepared under the direction of Lydia B. Edwards and Carroll E. Palmer with the assistance of Knut Magnus. Monograph Series No. 12. Geneva, World Health Organization, 1953. 307 pp. 15/—, $3.00, Sw. fr. 12.—

This monograph contains the results of an inquiry into the variable factors that affect BCG vaccination. The subjects investigated included the effects of long continued storage of vaccine, heat, light, changes in the technique of intracutaneous vaccination, variations in the preparation and concentration of the vaccine, mixtures of living and dead bacilli in different proportions, and the variability of vaccines prepared by different producers. Observations were made on more than 40,000 children in Denmark, Mexico, Egypt, and a rural part of southern India.

The most important feature of the monograph is the evidence presented to show the quantitative nature of tuberculin sensitivity. The authors recognize at least two kinds of tuberculin sensitivity. High-grade sensitivity (strong reactions to weak doses of tuberculin, i.e., 5-10 TU) constitutes the specific response to tuberculous infection. Low-grade sensitivity (small reactions to a weak dose of tuberculin which appear as larger reactions to a strong dose) constitutes a non-specific response. On epidemiological and statistical evidence it is postulated that low-grade sensitivity is due to something other than infection with *Mycobacterium tuberculosis* of the human or bovine variety. Such sensitivity would appear to be related to geographical factors. Should future investigations substantiate the hypothesis of low-grade sensitivity, it will become necessary to re-evaluate the results of past tuberculin testing surveys since it will no longer suffice to express tuberculin allergy in terms of percentage of positive reactors.

Investigation of the individual response to the injection of BCG revealed but little correlation between allergy and the vaccinal lesion. It could be shown that the size and severity of the local lesion were dependent upon the depth of injection, i.e., the deeper the injection, the larger the local lesion, but it was not possible to relate definitely age, sex, pre-vaccination sensitivity, or technique of vaccination to the variation in intensity of response. Surprisingly, a large source of variation was found to be a familial factor. This finding may prove of great significance, for should the capacity to develop allergy be related to susceptibility to tuberculous disease, BCG vaccination may be used to differentiate inherent susceptibility or resistance to tuberculosis.

Much of the other information contained in the monograph is now well known. Evidence is presented to show that BCG loses little allergenic potency when stored at low temperatures for a short period of time. Substantial decrement in allergenic potency occurs on prolonged storage, and the rate of deterioration increases as the temperature is raised. Exposure to sunlight causes a decrease in colony count and allergenic worth of vaccine suspensions. Although completely dead vaccine produces but low levels of allergy, the addition of only a small fraction of living organisms greatly potentiates the allergizing properties of the vaccine suspension.

The format of this monograph deserves comment. Following the introduction, each phase of the investigation is considered in a separate chapter.

Each chapter is subdivided into a descriptive portion, a critical discussion, and a summary. The volume ends with a general summary of the entire investigation, and 150 pages of minutely detailed appendices. A lucid style of writing makes this monograph deceptively easy to read, but the wealth of detail stamps it as one worthy of prolonged study, rather than casual perusal. It should prove of interest to immunologists, public health workers, and physicians who are primarily concerned with the preventive aspects of tuberculosis.

RUSSELL MILLER, JR.

JOURNAL OF RESEARCHES INTO THE GEOLOGY AND NATURAL HISTORY OF THE VARIOUS COUNTRIES VISITED BY H.M.S. BEAGLE. By Charles Darwin. Facsimile reprint of the first edition (1839). New York-London, Hafner Publishing Company, 1952. xiv, 615 pp. $7.50.

To most of us, the Darwinian concepts of evolution are so familiar that we give little thought as to how these ideas arose. We picture Charles Darwin in terms of the familiar portrait of an ancient and ageless, brooding genius looking with intuitive sureness to the inner purpose of living things.

In December 1831 a very human young Darwin shipped on board the *H.M.S. Beagle* as unpaid naturalist. He had thought to follow his father into the medical profession but had turned from it in horror. For no very obvious reason he decided on the church as a career and so went to Cambridge University. The courses he took there were of far less importance than the people he met and his own reading. He became fascinated with accounts of exploration such as the "Personal Narrative" of Humboldt; he fell under the influence of Henslow the botanist and through him received the offer of the appointment on board the *Beagle*. This somewhat brash young man in his early twenties, a very new B.A. in his pocket, sailed forth on His Majesty's 235-ton vessel. He returned five years and two days later, matured, and with world-wide experience. His Journal is the account of these five years.

Throughout the story of his voyage one is astonished at his boundless energy, his amazing knowledge of natural history, his perseverence as a collector. Everything he saw stirred his curiosity. A careful reading of this journal will show the beginning of almost all his famous works. Even where he has no answer he has an uncanny knack of laying his finger on the crucial point for which an answer is needed.

But we can feel much more at home with this Darwin of the Journal than with the ripened genius. As a Britisher, he is filled with indignation at slavery in foreign countries, forgetful that his own empire was only then in the process of abolition of slavery. Often he reached sweeping conclusions on pitiful evidence. Of New Zealand he stated: "It is not a pleasant place. Among the natives there is absent that charm . . . and the greater part of the English are the very refuse of society. Neither is the country itself attractive." He had seen a score of natives, survivors of the murderous Hongi wars, a handful of whalers, and perhaps twenty square miles of the country!

Several editions of this Journal exist and those commonly available are the later ones. The importance of this first edition lies in its timing. Within a year of his return from this voyage, Darwin, then in chronic ill health, had begun notes on *The Origin of Species*. By 1842 he had made a 35-page summary of his ideas; and his 230-page manuscript of 1844 contains all the essentials of his final work. Thus this first edition of the Journal is the last picture of his thoughts before they crystalized. Here we can study the development of his ideas, can sense his misery from seasickness and loneliness, and feel his rebellion at the goldfish-like privacy of his quarters—the end of the chart room in a vessel no bigger than a fishing boat.

Those interested in how ideas grow as well as in the perfected theory owe a debt to the Hafner Publishing Company for making this important edition available in facsimile.

ERNEST F. THOMPSON

FUNCTIONAL ENDOCRINOLOGY FROM BIRTH THROUGH ADOLESCENCE. By Nathan B. Talbot, Edna H. Sobel, Janet W. McArthur, and John D. Crawford. Cambridge, Harvard University Press, 1952. xxx + 638 pp., 220 figures and charts, 59 tables. $10.00.

CLINICAL ENDOCRINOLOGY. By Lewis M. Hurxthal and Natalija Musulin. Philadelphia, J. B. Lippincott Co., 1953. 2 vols. xiii + 1599 pp., 482 figures, 146 charts, 1 color plate.

There is probably no field of medicine in which progress has been so rapid, of recent years, as the field of endocrinology. As knowledge of the nature of the hormones and their mode of action has increased, new clinical techniques and theories have succeeded one another with bewildering rapidity. The clinician is sorely in need both of basic information and of a practical guide to its clinical application. These two books attempt to supply a *vade mecum* through the thickets of clinical endocrinology.

In spite of their common general purpose and the similarity of the field they cover, the two books are totally dissimilar. Talbot *et al.*, although concerned chiefly with endocrinology in childhood and adolescence, present a well-integrated review of experimental endocrine physiology, which would serve as an excellent background for anyone, regardless of the age of the patients he treats. As a matter of fact, the authors' interest in pediatrics does not prevent a fairly complete review of the entire field of endocrinology, even including pregnancy and the menopause.

The experience of Talbot's group in basic experimental endocrinology probably explains their interest in the rôle of the endocrines in the adjustments of the organism to the vicissitudes of normal living. The book is particularly notable for its exposition of the part played by the various glands of internal secretion in the growth, maturation, and defenses of the healthy organism. As a result, the book is, in a sense, a treatise on normal human endocrine function. Disease of the glandular system is treated, quite properly, as an alteration of normal physiology.

In view of the excellence of the basic material, *Functional Endocrinology* may, perhaps, be excused for presenting a somewhat restricted point of

view with regard to therapy. The authors frankly admit in the preface that the goal of a concise, clear, and self-sufficient text ". . . could be approached only at the price of considerable condensation of facts and of a certain amount of dogmatism. We have been forced to sift available information, to omit much historic, anatomic and histologic information, and to present in the main single rather than multiple theses." To some extent, therefore, the therapeutic recommendations represent the practices of the Massachusetts General Hospital group rather than a summary of all available approaches to a given problem. In each case, however, the authors present in detail the line of reasoning which has led them to adopt a certain therapeutic regimen, so that the reader can evaluate for himself the authors' conclusions.

Functional Endocrinology deserves particular praise for the excellence of its index and its Table of Contents which make the material in the book unusually accessible. Specific tests used in the physical diagnosis of the patient are described with highly commendable detail. Since the authors are writing for pediatricians, they have placed all dosage schedules on a uniform basis by referring them to the body surface area. Consequently, within reasonable limits the book can also be used as a reference for dosages applicable to adults.

In view of the general excellence of the book, it is unfortunate that the illustrations are reproduced so poorly.

Hurxthal and Musulin have presented a far more detailed and exhaustive work than Talbot *et al.,* but in certain respects their presentation is disappointing. The review of glandular physiology which precedes each clinical section is somewhat arbitrary, circumscribed, and occasionally even misleading, since the authors often select a single possible interpretation of the facts for presentation. The basic science section is uniformly devoid of actual data. On the other hand, this section is heavily documented, so that the inquiring reader can easily trace the basis of the authors' ideas. In contrast to the basic material, the clinical presentations are excellent in every respect. The authors have drawn freely from their collective vast experience, and present an enormous number of specific data regarding the clinical conditions under consideration. The generalities of the main text are copiously illustrated with case reports, photographs, diagrams, and tables. Moreover, since the organization of each chapter is uniform, and since the book is presented entirely in outline form, it is easy to find the answer to any particular question in a moment. The outline form of presentation, however, makes it impossible, at least for this reviewer, to browse through the book. A page or two of outline without any breaks for the exercise of the English language is difficult reading.

One might expect the outline form to tempt the authors to be arbitrary in their presentation, but this is not the case. In considering clinical problems where difference of opinion exists, the book sets forth all of the various viewpoints in a fair and dispassionate manner. Even in discussing such inflammatory topics as the therapy of hyperthyroidism or of diabetes mellitus, the authors succeed admirably in presenting the rationale and technique of therapeutic regimens which are certainly at wide variance with their own

usual practice. Indeed, there are some instances where one would have wished that the authors had taken a more definite and critical position.

Hurxthal and Musulin have, therefore, produced a book which serves one particular purpose, and serves it admirably. It is difficult to see how a more useful reference book for clinical use could have been compiled; but this is no book for the scholarly seeker after basic understanding, nor is it a book to be perused for pleasure. It has an extensive bibliography, which will be of aid to the student who is seeking an entry into the clinical literature, but it will be of only moderate help in respect to the literature relating to endocrine physiology. Moreover, it contains a fair number of textbooks and review articles which are useless as source material.

Both books, therefore, have their merits and their disadvantages. The Talbot book is particularly recommended for the student who is interested in a basic understanding of the rôle of the endocrines in normal and abnormal situations. The book by Hurxthal and Musulin is chiefly useful as a reference book which offers a tremendous store of clinical information in easily accessible form.

P. K. BONDY

BIOLOGIE DER GOETHEZEIT. By Adolph Meyer-Abich, Ed. Stuttgart, Hippokrates Verlag. Marquardt et Cie; Waltham, Mass., The Chronica Botanica Co., 1949. 302 pp. $5.50.

Writers dealing with the life of Goethe fall into two large groups, the protagonists and the antagonists. A comparatively small minority attempts to keep its objectivity with varying degrees of success. The reasons for this are obvious: Goethe's absorption in the period in which he was living, the avid interest which he had through his many contacts in all walks of life and in all branches of knowledge, and the stimulation of the changes of the times upon an active intellect. It is this latter factor which Meyer-Abich has tried to stress in his attempt to recreate something of the essence of the biological perspective of this period.

This recreation is necessary and is too frequently overlooked in attempting to evaluate Goethe by hindsight which unfortunately we must all use, for no one can relive the period with this man at the time he was reacting to it. We see only the dominant peaks, not the intermediate steps by which they arise. These peaks may be apparent to us although they never were to the individual to whom we now assign them.

In the introduction, Meyer-Abich has attempted to emphasize the difference between the ancient and modern points of view. He lists many points in our changing concepts and claims the Goethezeit as the bridge between. Unfortunately this transition is not an easy one to make, for there is no clear-cut point marking a complete change from static to dynamic, from vitalism to mechanism, or from the philosophical approach to the hypothesis and the hypothetical view of philosophy. Our thinking is never so precise that we can adopt even temporarily an absolute position unless we deny the variability of our so-called facts and assume an infallibility which for science is unbecoming and lacking in humility. Such an abrupt transition in concept

does not occur, actually, either in Goethe's times or in our own. His great advantage was both his actual and intellectual longevity that enabled him to incorporate in his thinking many of the gradual changes which were in the making and which his unincumbered scientific outlook made possible his seeing as simple explanations for rather broad generalizations.

Thus, in the study of plant pattern Goethe returned to Theophrastus, for uniformity of animal taxonomy to Aristotle. In neither case did Goethe know the details of the original generalization and his knowledge of phenomena was obtained by research without an understanding of the anticipatory observations. Nevertheless, these served as the basis of discussion with his many interesting companions through whom his scope of knowledge was widened and whose factual contributions did much to pinpoint his conceptual discrimination.

To recreate the period is to reincarnate the people in it. Since they live in their thoughts and writings, Meyer-Abich has made either an interpretation of their work or has given a selection of their writings which presents the reasoning and opinion of the individuals upon definite topics or concepts. One might question these selections but among the many that might have been chosen he presents six who certainly fit the thesis of Goethe as to the transitional period between the ancient and modern concepts in biology.

The selected six are George Forster, Alexander von Humboldt, Oken, Carus, von Baer, and Johannes Müller. Each marks a step in the transition from the concept of a naturalistic viewpoint of nature to the functional one of the organism. In some cases we know the value of their personal association through Goethe's appreciation given in his own words.

Meyer-Abich in the final note deals with the biological perspective as it affected Goethe's philosophy. It is certain that in the period the ideas of Kant were causing a considerable alteration in biological concepts. The Critiques of Reason and Judgment certainly permitted a temporary adjustment of purposefulness and mechanism which Goethe did not consider as paradoxical. Certainly Schelling, Fichte, and Hegel influenced the thinking of the time. The "holism" of Goethe was the outgrowth of these and other factors, most of them based on biological observation and thinking.

Meyer-Abich deserves thanks for bringing these materials together for us. For whatever our differences in interpretation may be, he has supplied a partial revisualization of a period of comparative tranquility and thought.

JOHN S. NICHOLAS

HUMAN LOCOMOTION AND BODY FORM, A STUDY OF GRAVITY AND MAN. By Dudley J. Morton, with the collaboration of Dudley Dean Fuller. Baltimore, The Williams and Wilkins Co., 1952. xii + 285 pp., 82 figs., 16 tables. $5.00.

This book consists of two parts. Part I, Underlying Principles of Adaptation, is to a large extent a revision and condensation of a previous book by Morton, *The Human Foot*. It deals at length with evolutionary considerations and concepts, some of them controversial. Several chapters

and strong emphasis are given to the rôle of gravity as a major designing agent of body structures. The author refutes the widely held idea that man gained his upright posture through an intermediate period of semi-erectness as may be observed in the gorilla and other anthropoid apes. This latter he regards as a reversion from the arboreally acquired vertical body posture of the primitive brachiating apes to terrestrial quadrupedism.

Several chapters are devoted to the bony channels of weight distribution within the foot in standing and running. The existence of an anterior transverse metatarsal arch is denied on the basis of experimental evidence of weight-bearing contact of the heads of all of the metatarsals with the ground. Primary importance is placed on the ligaments in maintaining the arches of the foot during long hours of standing. The muscles are credited with absorbing the sudden great stresses brought to bear on the foot during running and jumping.

In Chapters 13 through 16 the author presents his case for shortness of metatarsal I, and hypermobility of the first metatarsal segment, as the causes of the two dominant types of functional foot disorders. The first is characterized by pain centered in the metatarsal region with little evidence of structural deformity; the second is accompanied by pain of a more variable character and location, and by progressive pronation and flattening deformity. In Chapter 17 he makes vigorous rebuttal of the contrary conclusions drawn by Colonel R. I. Harris and Major Thomas Beath from the Canadian Army Foot Survey, which he labels a devastating arraignment of the "Morton thesis."

Part II of the book is a detailed, and at times highly mathematical, analysis of the walking stride, undertaken in collaboration with Dudley D. Fuller of the Mechanical Engineering Department of Columbia University. It is based to a great extent on barographic and motion picture studies applied to six young adult males. Among other things it considers the path of the body's weight center, foot contact, and synchronized actions and weight distribution, vertical and horizontal forces, metabolic aspects, and muscle function. The reproduction of sequential motion picture photographs provides very informative and interesting illustrative material. The last three chapters deal anew with evolutionary and philosophical considerations.

The book is rich with similies, but occasionally lacking in sufficient detail of experimental technique.

The bibliography lists Morton's previous book and the Canadian Army Foot Survey. For further material it refers the reader to the classified bibliographies of five other texts.

J. J. TRENTIN

SOIL MICROBIOLOGY. By Selman A. Waksman. New York, John Wiley and Sons, 1952. vii + 356 pp. $6.00.

This handsomely bound and attractively printed book proposes in the preface "to survey the nature and abundance of microörganisms in the soil. . . . It should . . . be considered . . . a textbook. . . . Appended to each

chapter . . . are references . . . where the student . . . can find up-to-date, concise statements. . . . My intention was to present here a broad outline of the subject." The preface is followed by an excellent historical introduction which gains much from the portraits of famous microbiologists.

Regrettably, not only is the book less informative than the preface leads one to expect, but it generally lacks up-to-date references. However, the chapter on "Associative and Antagonistic Effects" is a notable exception, reflecting Professor Waksman's long experience with antibiotics. While a brief textbook must naturally contain only a selected amount of information, much of the information contained in this book is unfortunately erroneous. To characterize protozoa by stating that they contain "protoplasm in a colloidal state" and that their most important constituents are "the complex proteins" is fatuous, and one wonders what the author had in mind when he assigned the formula $C_{36}H_{48}O_{30}N_4P_4$ to a "typical nucleic acid" which may contain "hexose or pentose." A tabulation of the nitrogen-fixing organisms includes such microbes as *Plectridium* (a genus found only in the historical section of Bergey's Manual), *Diplococcus pneumoniae,* and *Aerobacter aerogenes.* The latter two have never even been suspected of fixing nitrogen. On the other hand, the table omits such active nitrogen fixers as the photosynthetic bacteria (though the non-sulfur purples are given brief mention in the text). The oxidation of sulfur in the soil is attributed by the author exclusively to the thiobacilli and given an outmoded chemical formulation. The work of Guittonneau, who showed twenty years ago that most molecular sulfur in neutral soils is oxidized by numerous heterotrophic bacteria, is not even mentioned.

Space does not permit listing all sins of omission and commission. The reviewers wonder, however, why a large group of soil microörganisms of major importance, the algae, are dismissed in ten lines (and no references to the literature) which make no mention of their rôle in the economy of the soil. The reviewers regret that they do not consider this book useful or acceptable as a text.

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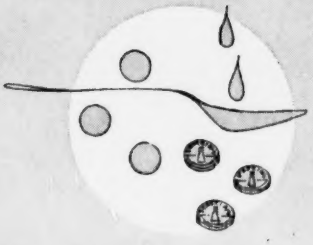

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